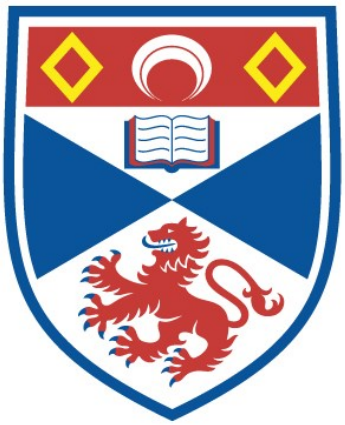
SOME NEW REACTIONS AND SOME NEW SYNTHESES OF LONG-CHAIN UNSATURATED ACIDS

Mohammad Golbar Hussain

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1973

In memory

of

my late father

SOME NEW REACTIONS AND SOME NEW SYNTHESES OF LONG-CHAIN UNSATURATED ACIDS

being a thesis

presented by

MOHAMMAD GOLBAR HUSSAIN, M.Sc.

to the

UNIVERSITY OF ST. ANDREWS

in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY

August 1973

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8019

DECLARATION

I hereby declare that this thesis is a record of the results of my own experiments, that it is my own composition and that it has not previously been presented in application for a higher degree.

The research work was carried out in the Department of Chemistry, University of St. Andrews, under the direction of Professor F.D. Gunstone, D.Sc., F.R.I.C.

CERTIFICATE

I hereby certify that Mohammad Golbar Hussain has completed twelve terms of research work under my supervision, has fulfilled the conditions of Ordinance 16 (St. Andrews) and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Research Supervisor

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SUMMARY

Part I. The preparation and reactions of long-chain acids containing sulphur

Much is known about long-chain hydroxy esters and the epoxides which can be derived from them but information about the corresponding sulphur compounds is sparse. Using various methods, methyl 12-mercaptostearate, methyl 9-mercaptostearate, methyl 12-mercapto-oleate, methyl 12-mercapto-elaidate, methyl 9(10)-mercaptostearate, methyl 12-hydroxy-9(10)-mercaptostearate, methyl 9-hydroxy-12(13)-mercaptostearate and 1-mercapto-octadec-4- and 5-ene have been prepared.

In reaction with sodium hydrogen sulphide without the rigorous exclusion of air the major product produced from methyl 9-mesyloxy-octadec-cis-12-enoate was methyl 9,12-epithiostearate. A similar result was observed with another δ-mercapto alkene (1-mercapto-octadec-4-ene) and with a δ-mercapto alkene (1-mercapto-octadec-5-ene), but not with a β-hydroxy alkene (methyl ricinoleate). For confirmation of these conclusions methyl 9,12-epithiostearate was synthesised by an independent and unambiguous route.

By an extension of the procedures applied for monomercapto esters, 1,2-dithiols (methyl erythro- and threo-9,10-dimercapto-stearate), a 1,3-dithiol (methyl 10,12-dimercaptostearate), and a 1,4-dithiol (methyl 9,12-dimercaptostearate) have been prepared from the corresponding dihydroxy compounds. The 1,3- and 1,4-dithiols are readily converted to cyclic epidisulphides by oxidation.

The infrared, NMR and mass spectral properties of these compounds have been investigated.

1,2-Epithiostearates (methyl cis and trans-9,10-epistearate) have been prepared by two different routes and their chromatographic (TLC, GLC) and spectroscopic (NMR, MS) behaviour studied.

Part II The synthesis of some C_{16} and C_{18} acids of geneal formula $CH_3(CH_2)_m(CH{=}CH)_nCO_2H$

Commercially available trienoic (△2, 4, 6) and tetraenoic acids (△2, 4, 6, 8) of medium chain length have been used successfully in the study of acyl-CoA synthetases of medium chain length specificity. But their activity with long chain acyl-CoA synthetases is rather low. The synthesis of C_{16} and C_{18} trienoic and tetraenoic acids has therefore been examined.

The triene esters were successfully prepared by the reaction of appropriate aldehydes with phosphonates prepared from methyl 6-bromo-hexa-2,4-dienoate and the tetraene esters by interaction of 2,4-dienals with a phosphorane. The C_{12}, C_{14} and C_{16} dienals were prepared from 1-methoxybut-1-en-3-yne by condensation with the appropriate saturated aldehyde.

The chromatographic (GLC) and spectroscopic (UV, IR, NMR, and MS) properties of these acids have been examined and some simple thiol esters made. Their biochemical use as reagents for the estimation of long-chain Co-A synthetases is being conducted elsewhere.

ABBREVIATIONS

GLC	-	Gas-liquid chromatography
DEGS	-	Diethyleneglycolsuccinate polyester
ApL	-	Apiezon L grease
ECL	-	Equivalent chain length*
NMR	-	Nuclear magnetic resonance
IR	-	Infrared
UV	-	Ultraviolet
MS	-	Mass spectrometry
TLC	-	Thin layer chromatography
Ag^{+}TLC	-	Silver ion thin layer chromatography
prep	-	preparative
PE	-	Mixture of petroleum ether (b.p. 40-60) and ether
TMS	-	Trimethylsilyl
TFA	-	Trifluoroacetyl
DMF	-	Dimethylformamide
DMSO	-	Dimethylsulphoxide
THF	-	Tetrahydrofuran

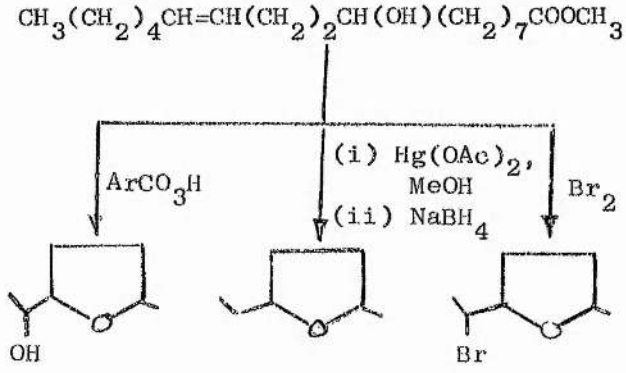
* The retention times of long-chain compounds are generally recorded as "carbon number" [170] or equivalent chain length (ECL) [167]. These indicate retention times in terms of the straight line log plot for the homologous series of methyl alkanoates.

Part I

The preparation and reactions of long-chain acids containing sulphur

INTRODUCTION

Recent work in this laboratory has provided examples of neighbouring group participation in the reaction of long-chain hydroxyalkenes, particularly of γ - and β-hydroxyalkenes. The reactions most extensively studied include epoxidation[1], oxymercuration[2] and bromination[3]. In these reactions a positive centre, generated by attack at the double bond, may interact with the nearby hydroxyl group to produce a cyclic ether. The following reactions of methyl 9-hydroxy-octadec-cis-12-enoate (γ -hydroxyalkene) furnishing 9,12-epoxystearate or its derivatives are typical:



The corresponding reactions of methyl ricinoleate (β-hydroxyalkene) gave no cyclic products although its trans isomer furnished cyclic ether in some of these reactions. These facts can be explained on the basis of the shape of the different reaction-intermediates which can either enhance or reduce the possibility of cyclisation.

The object of the present work was to prepare sulphur analogues of such γ - and β-hydroxyalkenes, and to study their reactions, examining, in particular, the possibility of forming cyclic compounds (episulphides).

Important synthetic methods for all major classes of

organic sulphur compounds were reviewed up to 1955 in the Houben-Weyl-Muller hand book[4] and this review was updated in 1972[5]. Additional general information is included in two specialised collections, each with a valuable appendix of reviews, books and symposia[6,7]; in Markley's five-part book on Fatty Acids[8], in two periodicals, published since 1966, containing much of synthetic relevance, "Quarterly Reports on Sulphur Chemistry" and "Mechanism of Reactions of Sulphur Compounds" (annual); in a monumental five-volume treatise[9]; and in three elegant volumes on Reagents in Synthesis that contain much sulphur chemistry[10,11,12]. The appearance of a continuing review "Organic Compounds of Sulphur, Selenium, and Tellurium" (Chemical Society, Specialist Report, ed. D.H. Reid) is welcomed.

Recent literature shows little evidence of activity either in the search for new methods of synthesis or in the investigation of reactions of long-chain sulphur compounds. Early literature describing sulphur derivatives of fatty materials is vague. For example, a patent[13] issued in 1939 describes reactions between various unsaturated glycerides and sulphur in the presence of iodine. The products of these reactions apparently contain sulphur, and claims were made that they are useful insecticides. However, no structures were proposed for these materials and no properties were described.

A significant contribution to the sulphur chemistry of fatty compounds was made in 1939 by Ralston and co-workers[14] who prepared a series of thiol esters of lauric, myristic, palmitic, stearic and oleic acids. Prior to this work, thiol esters of long-chain acids were considered to be unstable.[15] Thiol esters

can not be prepared by direct esterification of acids and thiols, but are conveniently obtained by interaction of an acyl chloride with an alkane thiol or benzene thiol.

$$RCOCl + R' SH \longrightarrow RCOSR'$$

The stability of thiol esters is evidenced by the fact that they may be distilled under diminished pressure without excessive decomposition. Other investigations[16,17] later synthesised a variety of long-chain thiol esters, and confirmed the earlier findings regarding their stability.

1. Preparation of monomercapto compounds

(i) Primary alkane thiols have been prepared from alkyl chlorides by reaction with sodium trithiocarbonate

$$RCl + Na_2CS_3 \longrightarrow R\cdot SCS_2Na \xrightarrow{H^+} RSH + CS_2$$

This method has also been used for the preparation of primary dithiols[18].

(ii) Another method of preparing thiols reported by Stevens[19] and developed by Johnson and Sprague[20,21] involves the alkaline hydrolysis of S-alkylisothiouronium salts, formed by the direct action of thiourea and halogen acids on alcohols.

$$ROH + H_2NCSNH_2 + HX \longrightarrow RSC(=\overset{+}{N}H_2)NH_2\ \bar{X} \xrightarrow{NaOH} RSH$$

Several alkane thiols have also been prepared by treating the appropriate alkyl bromide with thiourea[22,17].

(iii) The reaction of alkyl halides or sodium alkylsulphates with an alkali-metal hydrosulphide is another well known route[23] to mercapto compounds.

$$RX + NaSH \longrightarrow RSH + NaX$$

(R can be a primary, secondary, or tertiary alkyl group; X can be Cl, Br, I, $-OSO_3Na$ etc)

A literature search disclosed that the only secondary long-chain thiol which has been prepared by this route is 2-mercapto-stearic acid[24].

(iv) Another route to the preparation of thiols, reported by Owen and Chapman[25], is the acid or alkaline hydrolysis of acetylmercapto compounds formed by the action of potassium thiolacetate with mesyloxy compounds.

$$RCH(OMs)R' \xrightarrow{KSAc} RCH(SAc)R' \xrightarrow{H^+} RCH(SH)R'$$

During acid or alkaline hydrolysis some dimer may accompany the desired mercaptan. Eliel and Hutkins[26], reported that the formation of such dimer could be avoided by deacetylating the acetylmercapto compound in a reducing medium (methanolic hydrochloric acid and zinc amalgam with the use of degassed water during the recovery of the product).

(v) Thiols and their derivatives may be obtained by radical-catalysed addition of SH compounds to alkenes. The most common sulphur compounds used in this reaction are thiol-acetic acid[27,28], mercaptoacetic acid[29], and 3-mercaptopropionic acid[30]. The acetylmercapto product formed in the first reaction

$$-CH=CH- \xrightarrow{CH_3COSH} -CH_2CH(SAc)- \longrightarrow -CH_2CH(SH)-$$

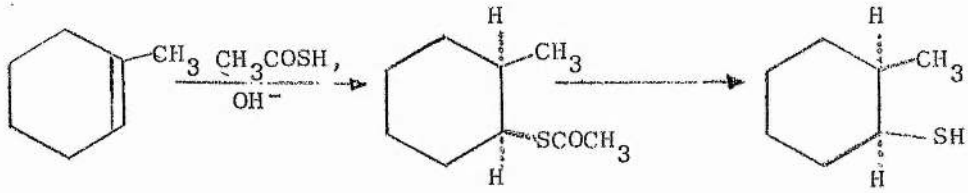
$$-CH=CH- \xrightarrow{SHCH_2COOH} -CH_2CH(SCH_2COOH)-$$

$$-CH=CH- \xrightarrow{SHCH_2CH_2COOH} -CH_2CH(SCH_2CH_2COOH)-$$

can be hydrolysed to the corresponding mercapto compound.

Liberation of the mercapto compound is frequently followed by dimerisation to a disulphide (2RSH ⟶ RSSR) but this can be prevented or limited under reducing conditions as described above.

Using 1-methylcyclohexene and 1-methylcyclopentane these additions have been shown to be largely <u>trans</u>[31].



(85%)

Monosubstituted alkenes give mainly a single product[28], disubstituted alkenes generally give a mixture of two products[27]. Some examples are given:

$$CH_2{=}CH(CH_2)_8COOH \xrightarrow[\text{peroxides}]{CH_3COSH} CH_3COS(CH_2)_{10}COOH \xrightarrow[H^+]{HOH} HS(CH_2)_{10}COOH$$

11-mercapto-undecanoic acid

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOCH_3 \xrightarrow[UV]{CH_3COSH}$$

$$CH_3(CH_2)_{8(7)}CH(SAc)(CH_2)_{7(8)}COOCH_3 \xrightarrow[NaOMe]{CH_3OH}$$

$$CH_3(CH_2)_{8(7)}CH(SH)(CH_2)_{7(8)}COOCH_3$$

When dithiols are added to such compounds as 11-undecanoic acid in the presence of peroxides the products contain two sulphur atoms[32]

$$HS(CH_2)_nSH + 2CH_2{=}CH(CH_2)_8COOH \xrightarrow{\text{Peroxides}}$$

$$HO_2C(CH_2)_{10}S(CH_2)_nS(CH_2)_{10}CO_2H$$

alkane bis-(11)-thioundecanoic acid

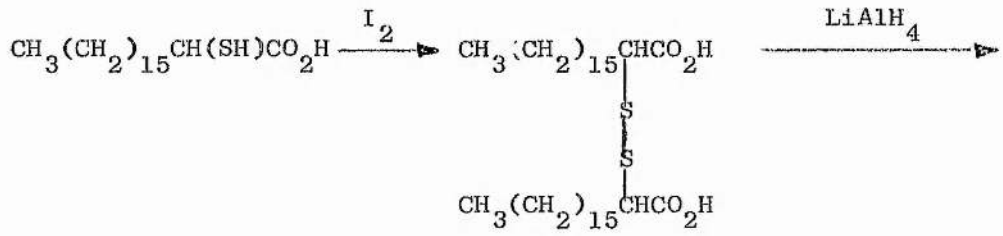
These dicarboxylic acids, like the simpler compounds, are readily converted to the corresponding sulphones and esters[32].

Sulphur-containing polymers have been prepared from long-chain dithiodicarboxylic acids by heating them with hexamethylene diammine to produce polyamides[33]. These polyamides (mol. wt. 11,000-20,000) have been converted into fibres.

The addition of sulphur and sulphur-chlorine compounds to unsaturated oils has been practised for some time in the production of commercial materials suitable as lubricants, plasticisers, and rubber-like products[34,35].

2. Reaction of monomercapto compounds

Monomercapto compounds can be easily oxidised to form dimers. Oxidants like iodine and peroxides have been used successfully[36,37]. The regeneration of the monomercapto compounds can be accomplished by reduction with lithium aluminium hydride[38,39,40]. Both 2-mercaptostearic acid[27] and methyl 9(10)-mercaptostearate[27] react in this way.



$CH_3(CH_2)_{15}CH(SH)CH_2OH$

The thiolacetate, produced by the radical addition of thiolacetic acid to terminal olefins, can be oxidised to the sulphonic acid[41] with peracetic acid generated *in situ* from hydrogen peroxide and acetic acid

3. Preparation of dimercapto compounds

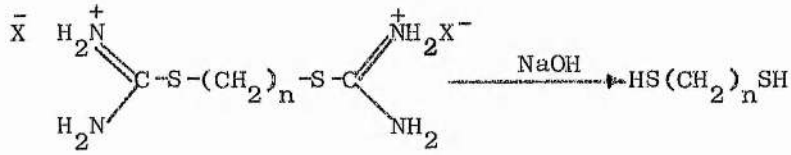
The chemistry of dithiols, although sporadically studied since the latter part of the last century, has attracted more widespread attention during the past two decades. A stimulus to work in this field was provided by the discovery, during the second world war, that some simple dithiols, in particular 2,3-dimercaptopropanol (British Anti-Lewisite; BAL) were effective antidotes for Lewisite and other arsenicals. More recently, the identification of a trimethylene disulphide system (the oxidised form of a 1,3-dithiol) in gliotoxin and in the biologically important lipoic acid has aroused much interest.

Aromatic dithiols are readily available by reduction of disulphonyl chlorides[42-53]. The majority of aliphatic dithiols have been prepared directly or indirectly from the corresponding dihalides by a simple extension of the procedures described for the monomercaptans. When both groups are primary the reactions occur readily and usually in good yield.

(i) Compounds in the series $HS(CH_2)_nSH$ are obtained by conventional methods, such as direct interaction of the dihalide with an alkali-metal hydrogen sulphide[54-57]

$$X(CH_2)X \xrightarrow{NaSH} HS(CH_2)_nSH$$

(ii) The dithiols have also been prepared from the corresponding diisothiouronium dihydrohalide salt[55,58]



(iii) Another conventional way of preparing dithiols is by hydrolysing the bisthiolacetal[57] produced from dihalide by

reaction with potassium thiolacetate.

$$X(CH_2)X \xrightarrow{KSAc} SAc(CH_2)_nSAc \longrightarrow HS(CH_2)_nSH$$

Reaction of a toluene-p-sulphonate or methanesulphonate with potassium thiolacetate is an alternative to the use of a dihalide, and provides a route for the conversion of hydroxyl into thiol by the sequence:

$$ROH \xrightarrow{OMs\ (C_6H_5SO_2Cl)} ROMs \xrightarrow{KSAc} RSAc \xrightarrow{H^+} RSH$$

This is of potential value in cases where the corresponding halide is not easily available. Primary groups react very readily[25,58,59], but secondary ones show considerable difference in behaviour[25,58].

(iv) Conversion of dihalide into cyclic sulphide followed by reduction with sodium in liquid ammonia has been used to prepare dithiols[60]

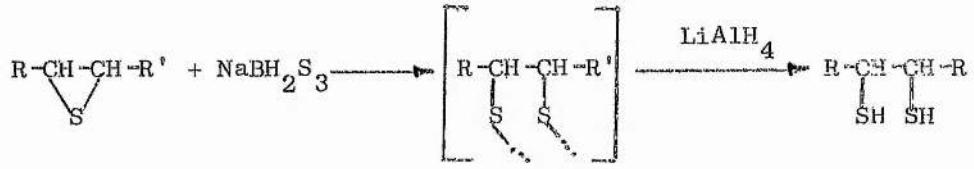
$$X(CH_2)_nX \xrightarrow{KSH} \underset{\lfloor\_\_\_\_\rfloor}{S(CH_2)_nS} \xrightarrow[\text{liquid } NH_3]{\text{Na in}} HS(CH_2)_nSH$$

(v) It has also been reported that cyclic trithiocarbonates can be prepared from epoxides or episulphides. Poor yields of

dithiols are obtained on hydrolysis[61,62] and better results can be obtained by reduction with lithium aluminium hydride[63]. Claims have been made that this procedure is particularly useful in the carbohydrate field, and for the preparation of vicinal di-secondary thiols

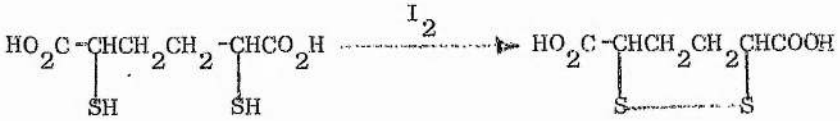
(vi) A very recent report[64] on the preparation of 1,2-dithiols

from the corresponding episulphides involves reaction with sulphurated sodium borohydride followed by reduction of a polymeric intermediate with lithium aluminium hydride.



4. Reactions of dimercapto compounds

Oxidation of 1,2-dithiols with bromine or iodine gives a product which is probably[54] mainly cyclic tetrasulphide, though higher polymers may also be formed. Other dithiols can undergo intramolecular radical cyclisation. Thus αα'-dimercapto adipic acid readily yields a monomeric cyclic sulphide[65] (1) and the biologically important lipoic acid (the disulphide from 6,8-dimercapto-octanoic acid) is readily prepared by oxidising[66] the 6,8-dimercapto-octanoic acid.



αα'-dimercapto adipic acid

(1)

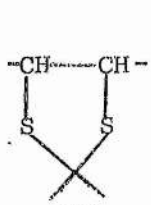
6,8-dimercapto-octanoic acid

(2)
lipoic acid

(1,2-dithiolane-3-valeric acid)

The nature of the product formed by condensation of a dithiol with an aldehyde or a ketone depends on the relative positions of the thiol groups. Aliphatic 1,2- and 1,3-dithiols give

1,3-dithiolanes (3) and 1,3-dithianes (4) respectively[54,56,60,67,68].



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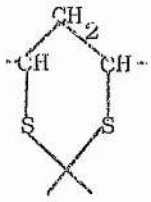
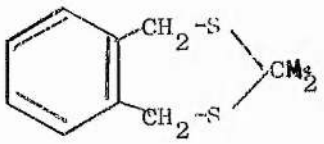
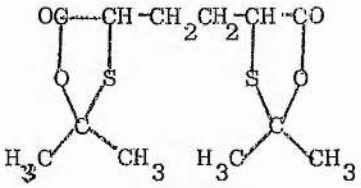


(4)

There appears to be no record of the behaviour of a simple aliphatic 1,4-dithiol though o-xylene dithiol gives the seven-membered ring[69] compound (5), whilst αα'-dimercapto adipic acid condensed with acetone to yield the dilactone (6)

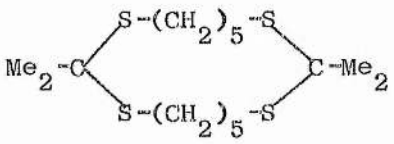


(5)

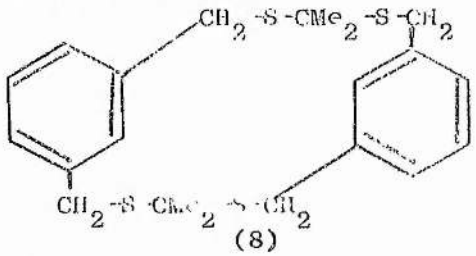


(6)

With more atoms between the thiol functions, tetrathio macrocycles are formed, some of which have been known for a surprisingly long time. Fifty years ago, Autenrieth[70] described two sixteen-membered rings (7) and (8) derived respectively from pentane-1,5-dithiol and m-xylene dithiol and also the eighteen-membered ring (9) from p-xylene dithiol.



(7)



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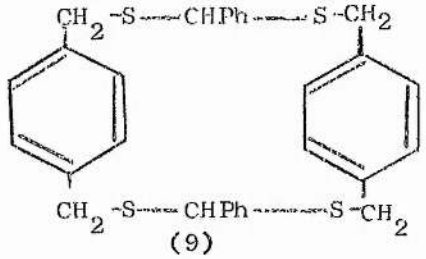
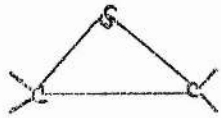


(9)

More recent examples include analogues of (7) containing 20, 24 and 26-ring-atoms, derived from heptane, nonane, and decane-α, ω-dithiols[71]. Linear polymers are also formed. The condensation of ethane-1,2- or propane-1,3-dithiol with aldoses[72-76] and with steroid ketones[77] is now well known.

Epithio compounds

Thirane is the official term for three-membered rings comprising one sulphur atom.



Other designations used for these compounds include olefin sulphides, alkene sulphides, episulphides, etc. However, when the ring compound is more than three-membered the prefix epithio attached to the name of the saturated hydrocarbon derivative may be more convenient nomenclature.

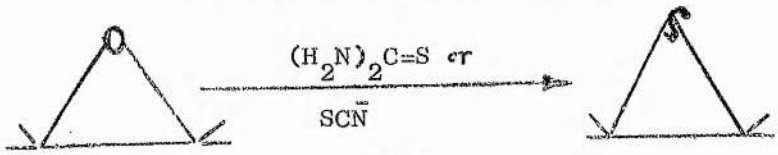
Thirane compounds have been known for a relatively short period of time. In 1916 Staudinger and Pfenniger[78] mentioned the synthesis of tetraphenylethylene sulphide and its thermal decomposition to tetramethylethylene and sulphur; a more detailed description of this reaction was published in 1920[79]. In the same year Delépene[80] prepared ethylenesulphide, the first pure aliphatic thirane and recognised the importance of this group of compounds as reactive substances suitable for a variety of reactions. Becuase of technical interest of these compounds Dachlauer and Jackal (1934) discovered a method for the synthesis of thiranes from epoxides by reaction with alkali thiocyanates or with thiourea[81]. Most of our present knowledge of the chemistry of thiranes is due to studies conducted by Culvenor and Davies

between 1949 and 1952[82-87].

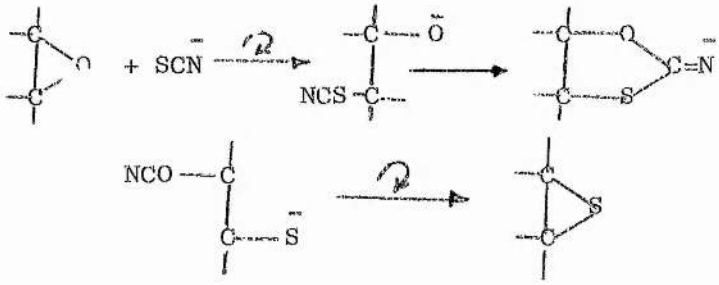
In 1951, Tarbell and Harnish[88], presented the first summarising review on the ring-opening reactions of thiranes; in 1953 the first general review on thiranes was published in Japanese[89]. A review in German was given by Schönberg in 1955[90]; a Russian review appeared in 1957[91]. Short summaries on the chemistry of thiranes have been published by Reid[92] and Kaufman and Schickel[93]. A review covering the literature on the preparation, properties and reactions of thiranes up to 1964 has been published by M. Sander[94]. An excellent review of thiranes, particularly of long-chain compounds has been reported by G. Maerker[95].

5. The preparation of 1,2-epithio compounds

(i) The most important method for the synthesis of thiranes is the reaction of epoxides with thiourea or inorganic thiocyanates as described for the first time in a patent specification[81]



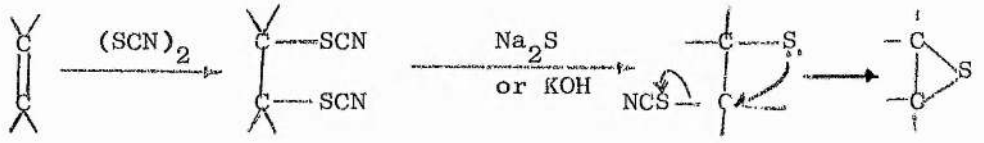
According to several authors[96-99], the reaction of alkene oxides with thiocyanates proceeeds by the following mechanism:



The entire reaction is accompanied by Walden inversion at both of the two C atoms of the ring, so that L(-)-2-butene sulphide results from D(+)-2-butene oxide[99].

A similar reaction mechanism has been proposed for the reaction with thiourea[96,85].

(ii) While it is often useful to employ epoxides as intermediates in the synthesis of epithio acids, episulphides can also be prepared from the unsaturated fatty acids by more direct routes. Vicinal dithiocyanato fatty acids, obtained by the addition of thiocyanogen to the olefins are converted to episulphides upon treatment with sodium sulphide or potassium hydroxide in accordance with the equation:



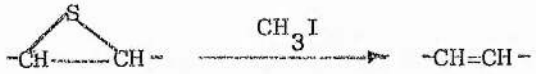
cis threo cis

The same results are obtained on addition of thiocyanogen chloride to the double bond and treatment of chlorothiocyanate with base.

6. Reactions of 1,2-epithio compounds

A recent review of ethylene sulphide and other low molecular weight terminal episulphides[94] indicates that many of the reactions of these compounds are similar to those of epoxides. All of the reactions of episulphides occur with ring opening.

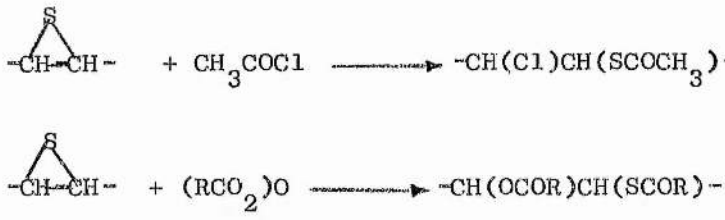
The episulphides are readily converted to olefins either thermally or chemically by organophosphorous or organometallic compounds and desulphurisation often occurs stereospecifically.



trans trans

The sulphur heterocycles react more readily than their oxygen

analogue with acyl halides or with carboxylic anhydrides.



Any oxidation of the episulphides entails ring opening[84]. Thus, the carboxysulphonic acids were isolated on oxidation of ethylene sulphide with nitric acid[100]. Hydrogen peroxide reacts vigorously with ethylene sulphide[84]; 2-hydroxypropanesulphonic acid, $CH_3CH(OH)CH_2SO_3H$, was obtained from propene sulphides[102]. Oxidation of ethylene sulphide with perbenzoic acid or dibenzoyl peroxide furnished insoluble polymers, probably poly(ethylene sulphone)[101].

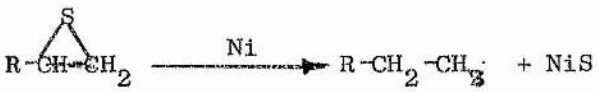
Reduction of aliphatic alkene sulphides with lithium aluminium hydride yields secondary mercaptans[103]:

The reduction of cyclohexene sulphide[103,104] and carbohydrate thiranes[105] with lithium aluminium hydride was also reported. By-products of the reduction are solid polymers and sulphur containing lipids, but not hydrogen sulphide[106].

Relatively resistant to reduction by lithium aluminium hydride are steroidal thiranes and some higher thiranyl carboxylic esters. Such esters may be reduced to the alcohols without effecting the thirane group[107,108]. At higher temperatures desulphurisation occurs with the formation of olefins[108,109,110].

Reductive desulphurisation may be accomplished with Raney

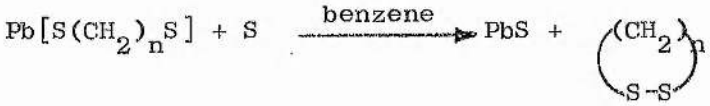
nickel in ethanol[105,108,111]



7. Epidithio compounds

An excellent report[112] on the preparation and properties of lipoic acid gives valuable information about the preparation of related epidithio compounds. Most epidithio compounds have been prepared by the oxidation of appropriate dithiols.

A very recent report[113] on the synthesis of 5-7 membered cyclic disulphides provides a further example for the preparation of such compounds.

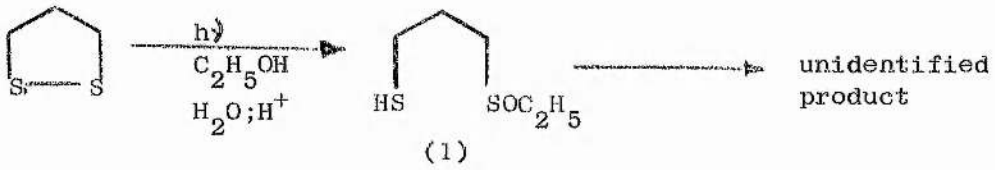


Lead dithiolates, obtained by the almost instantaneous and quantitative reaction of dithiol with an aqueous solution of lead acetate, react readily with sulphur to give lead sulphide and the corresponding cyclic disulphide. Other oxidants like selenium, chlorine and iodine are also effective.

8. Reactions of epidithio compounds

Epidisulphides can be reduced by lithium aluminium hydride to the corresponding dithiols.

Barltrop[114] et al observed that photolysis of 1,2-dithiolane in neutral solution with near ultraviolet light resulted in polymerisation. Photolysis in acidified ethanol did not lead to polymerisation, but the 1,2-dithiolane ring was destroyed.

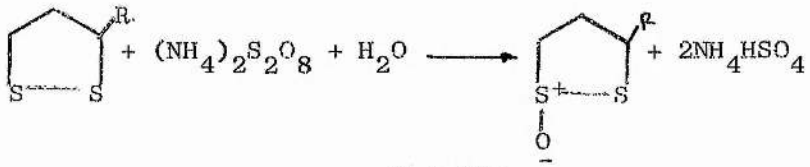


(1)

Although the nature of this reaction has not been elucidated, it has been suggested that the dithiyl radical produced by photolysis reacts with the solvent to produce an unstable thiol sulphenate (1), which undergoes further change to give an unidentified product.

It has been reported that a sulphoxide was obtained during the isolation of lipoic acid (1,2-dithiolane-3-valeric acid), due to the tendency of this substance to undergo oxidation. Such conversion could be effected by mild oxidising agents, such as t-butylhydroperoxide. It has not been established which sulphur atom is in the oxidised state, but there is some evidence that the sulphur atom attached to C(8) in the octanoic acid was involved.

Barltrop et al[114] found that 1,2-dithiolane was rapidly photo-oxidised to a monosulphide in the presence of zinc tetraphenylporphin. Comparable results were obtained with ammonium persulphate as the oxidising agent. The course of the latter reaction may be presented as follows.

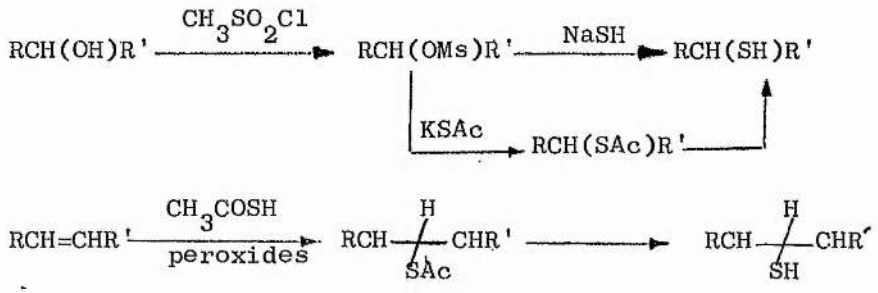


A kinetic study of this reaction[115,116] revealed that the rate of oxidation of lipoic acid was approximately 30 times as fast as that of the six-membered cyclic disulphide, 1,2-dithiane-3-butyric acid. A seven-membered cyclic disulphide, 1,2-dithiepane-4-carboxylic acid, was barely attacked by persulphate, and an open-chain disulphide, n-propyldisulphide, was unstable to persulphate under the conditions employed. These results provide additional evidence of the unique reactivity of the 1,2-dithiolane ring.

DISCUSSION

1. Preparation of monomercapto C_{18} esters

As already indicated in the Introduction very little is known about monomercapto C_{18} acids and esters. After examining several procedures we have found three methods to be of value. Two require the corresponding hydroxy ester as starting material and the third starts from the corresponding alkenoate. These are summarised in the following equations:



Preparations by these three methods will be discussed first followed by comments on their relative merits.

1.1 Reaction of mesyloxyesters with sodium hydrogen sulphide

The hydroxyl group is not a good leaving group so that reactions of alcohols with nucleophilic reagents (particularly in the non-acidic media) are not very useful as synthetic procedures. This difficulty can be overcome however by conversion of the OH group to sulphonate esters such as the mesyloxy (CH_3SO_2O) or tosyloxy ($P\text{-}CH_3C_6H_4SO_2O$) derivatives. These are known to be excellent leaving groups and to take part in nucleophilic substitution processes.

When a mesyloxy ester was treated with sodium hydrogen sulphide in dimethylformamide solution the product contained the desired mercapto ester along with some of its dimer, some unreacted mesylate, and some alkenoate resulting probably from a competitive elimination

process. Chromatographic separation gave a fraction containing both the mercapto ester and the alkenoate and these could only be separated after acetylation of the mercapto ester.

The products of these reactions were generally identified by a combined study of the infrared, NMR and mass spectra of the mercapto esters and of their acetyl and trifluoroacetyl derivatives prepared by reaction with acetyl chloride and trifluoroacetic anhydride respectively.

1.1a Methyl 12-mercaptostearate

Methyl 12-mesyloxystearate was prepared from methyl ricinoleate (isolated from castor oil) by hydrogenation followed by reaction with methanesulphonyl (mesyl) chloride in pyridine. This ester was heated at 100°C with sodium hydrogen sulphide in dimethylformamide[117]. TLC examination of the reaction product at intervals of 30 minutes showed that the starting material had almost entirely disappeared after 30 minutes and that prolonged reaction resulted in increasing amounts of more polar materials. Reaction also occurred fairly quickly at room temperature and optimum yields were obtained after 24-30 hours. In general our substitution reactions were effected at room temperature for 24-30 hours.

A more detailed examination of the products showed them to be mixtures of methyl 12-mercaptostearate, methyl octadecenoate, a dimer of the mercapto ester, starting material and some unidentified polar material, thus:

	Fraction (% wt)			
Reaction condition	12SH 18:0 + 18:1	Dimer	Unreacted starting material	Unidentified product
20° (24-30 hr)	50-60	20-30	5	10
100° (3-4 hr)	30	30-35	-	30-40

The product of the reaction at room temperature was examined in detail.

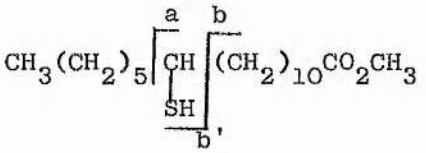
Fraction A (50-60%) showed two peaks on GLC of ECL 18.5 (10%) and 23.2 (90%). These components were only separated (TLC) after acetylation. The less polar and smaller subfraction (10%) showed a single peak of ECL 18.5 on GLC and was considered to be a methyl octadecenoate.

The larger subfraction (90%, ECL 25.4) was shown to be methyl 12-acetylmercaptostearate. Hydrolysis and remethylation gave methyl 12-mercaptostearate (ECL 23.2) which could be acylated to the acetylmercapto and the trifluoroacetylmercapto ester (ECL 21.5). The evidence of these structures is set out below:

(i) The infrared spectra of the acetylmercapto and trifluoroacetyl-mercapto esters contained strong absorption bands due to carbonyl stretching at 1685 ($SCOCH_3$) and at 1700 ($SCOCF_3$) cm^{-1} respectively. The NMR spectrum of the acetylated ester showed a three-proton singlet at 7.72τ ($SCOCH_3$). These spectral properties were generally useful in identifying our mercapto products which themselves contain no characteristic and distinctive absorption.

(ii) Subjected to Mozingo hydrogenolysis (Raney nickel) the mercapto ester gave methyl stearate (identity on TLC and GLC).

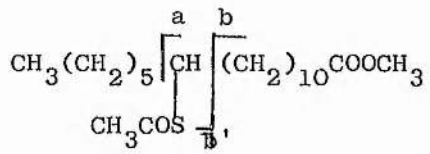
(iii) The mass spectrum of the mercapto ester showed peaks at 330 (M, 12), 299 (M-31, 6), 298 (M-32, 6), 297 (M-33, 12), 296 (M-34, 6), 265 (M-65, 21), 264 (M-66, 36), 222 (M-108, 9), 213 (a-32, 12), 200 (b+1, 4), 199 (b, 4), 131 (b', 5) and 74 ($C_3H_6O_2$, 64) along with peaks of the series $(CH_2)_nCOOCH_3$ (59-199), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167)



These peaks are in line with the expected structure but only those at m/e 213 (a-32), 200 (b+1), 199 (b) and 131 (b') indicate the position of the mercapto group. The fragmentation pattern

indicates that the molecular ion may lose OCH_3 (31), CH_3OH (32), HS (33) or H_2S (34) or two of these fragments. The peak at 222 (M-108) is thought to result from the loss of 74 ($CH_2=C(OH)OCH_3$) and 34 (H_2S) mass units.

(iv) The mass spectrum of the methyl acetylmercapto ester contained peaks at 372 (M, 3), 330 (329+1, 22), 329 (M-43, 100), 299 (M-73, 30), 298 (M-74, 20), 297 (M-75, 100), 296 (M-76, 13), 265 (M-107, 30), 264 (M-108, 50), 222 (M-150, 11), 213 (a-74, 19), 200 (b+1, 3), 199 (b, 5) and 131 (b'-42, 15) along with peaks of the series $(CH_2)_nCOOCH_3$ (59-199), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).



Many of these ions readily lose CH_3CO (43), CH_3O (31), CH_3OH (32), sulphur compound (33 and 34) or $CH_2=C(OH)OCH_3$ (74) and combinations of these. The spectrum contains the same fragments at m/e 213, 200, 199 and 131 as are observed in the unacetylated mercapto ester.

Band B (20-30%) which showed no peak on GLC was considered to be the dimer of methyl 12-mercaptostearate. Mozingo hydrogenolysis gave methyl stearate. It was unchanged on treatment with sodium borohydride but reduction with lithium aluminium hydride gave methyl 12-mercaptooctadecanol identical with that obtained by lithium aluminium hydride reduction of methyl 12-mercaptostearate on the basis of the infrared spectrum of the alcohol (OH stretching at 3500 cm^{-1}). Both samples of the mercapto alcohol gave identical bis TFA derivatives (ECL 21.0) with C=O stretching at 1700 ($SCOCF_3$) and at 1780 ($OCOCF_3$) and characteristic NMR signals at 5.75 (t, 2H, $-CH_2OCOCF_3$) and at 6.38τ (m, 1H, $-CH(SCOCF_3)-$).

Methyl 12-mercaptostearate was oxidised to the dimer when

treated with iodine and the monomeric form was regenerated as the alcohol by lithium aluminium hydride reduction.

Band C (5%) was probably unreacted mesylate on the basis of its behaviour on TLC and GLC [mainly a peak at 18.5 (methyl octadecenoate) resulting from on-column decomposition of the mesyloxy ester].

Band D (~10%) gave no peak on GLC even after methylation and was not examined further.

1.1b Methyl 9-mercaptostearate

Methyl 9-hydroxyoctadec-cis-12-enoate was obtained from the mixed acids of Strophanthus sarmentosus seed oil by partition between petroleum and 80% aqueous methanol[118]. The crude acid after methylation (methanolic sulphuric acid) was purified by column chromatography before being hydrogenated over 10% palladium on charcoal as catalyst. The product was reduced with sodium borohydride to give methyl 9-hydroxystearate and finally purified by column chromatography.

Methyl 9-mesyloxystearate, prepared from the hydroxy ester by reacting with methanesulphonyl chloride in pyridine, was kept overnight at room temperature with sodium hydrogen sulphide in dimethylformamide and then separated into four fractions by prep TLC.

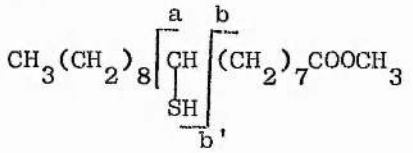
Band A (65%) which had ECL of 18.6 (12%) and 23.2 (85%) was separated after acetylation into two subfractions. The less polar subfraction (ca 10% , ECL 18.6) was presumably the monoenoic ester formed by an elimination process. The larger subfraction (85%, ECL 25.5) was considered to be methyl 9-acetylmercaptostearate. Hydrolysis and re-methylation gave methyl 9-mercaptostearate (ECL 23.2) which could be acetylated to the acetylmercapto (ECL 25.5) and the trifluoroacetyl-mercapto ester (ECL 21.4) These structures are based on the following

evidence:

(i) The infrared spectrum of the acetylmercapto and the trifluoroacetylmercapto ester showed strong absorption bands due to carbonyl stretching at 1685 ($SCOCH_3$) and at 1700 ($SCOCF_3$) cm^{-1} respectively. The NMR spectrum of the acetylated ester showed a three-proton singlet at 7.72τ ($SCOCH_3$).

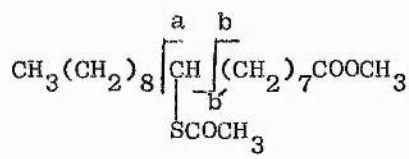
(ii) Subjected to Mozingo hydrogenolysis (Raney nickel) the mercapto ester gave methyl stearate (identity on TLC and GLC).

(iii) The mass spectrum of the mercapto ester showed peaks at 330 (M, 9), 299 (M-31, 7), 298 (M-32, 6), 297 (M-33, 20), 296 (M-34, 6), 266 (M-64, 9), 264 (M-65, 16), 264 (M-66, 40), 222 (M-108, 10), 173 (b', 6), 171 (a-32, 12), 158 (b+1), 4), 157 (b, 4), 141 (b'-32, 5), 74 ($C_3H_6O_2$, 72) and member of the series $(CH_2)_nCOOCH_3$ (59-143), C_nH_{2n+1} (57-169), and C_nH_{2n-1} (55-167).



These peaks are in line with the expected structure and the peaks at 173 (b'), 171 (a-32), 158 (b+1) and 157 (b) indicate the position of the mercapto group at C(9). The fragmentation pattern suggests that the molecular ion may lose OCH_3 (31), CH_3OH (32), SH (33) or H_2S (34) or two of these fragments. The peak at 222 (M-108) may result from the loss of 74 (CH_2=C(OH)OCH_3) and 34 (H_2S) mass units.

(iv) The mass spectrum of the acetylmercapto ester contained peaks at 372 (M, 1), 330 (329+1, 8), 329 (M-43, 39), 299 (M-73, 13), 298 (M-74, 9), 297 (M-75, 35), 296 (M-76, 13), 265 (M-107, 13), 264 (M-108, 17), 222 (M-150, 7), 173 (b'-42, 7), 171 (a-74, 13), 158 (b+1, 2), 157 (b, 4) and members of the series $(CH_2)_nCOOCH_3$ (59-143), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).



Some of these ions lose CH_3CO (43), CH_3O (31), CH_3OH (32), sulphur compounds (32 and 34) or $CH_2=C(OH)OCH_3$ (74) mass units and combinations of these. The spectrum contained the significant fragments at 173, 171 and 157 already observed in the unacetylated mercapto ester.

Band B (25%) gave no peak on GLC and was probably the dimer of methyl 9-mercaptostearate. Mozingo hydrogenolysis furnished methyl stearate (identity on TLC and GLC). A sample of methyl 9-mercapto stearate oxidised by iodine showed polarity identical with band B (TLC) and gave no peak on GLC.

Band C (4%) was probably unreacted mesylate on the basis of its behaviour on TLC and GLC [mainly a peak at 18.5 (methyl octadecenoate) resulting from the on-column decomposition].

Band D (ca 5%) gave no peak on GLC even after methylation (boron trifluoride/methanol) and was not examined further.

1.1c Methyl 12-mercapto-oleate

Methyl 12-mesyloxyoleate, obtained from methyl ricinoleate, was reacted with sodium hydrogen sulphide in dimethylformamide at room temperature for 24-30 hours and the product was separated into four bands on prep TLC.

Band A (60-65%) showed four peaks on GLC (ECL 18.8, 20.0, 20.3 and 23.7). These components were only successfully separated after acetylation. The less polar and smaller subfraction (12%) showed GLC peaks of ECL 18.8, 20.0, and 20.3 and was considered to be isomeric methyl octadecadienoates.

The larger subfraction (85%, ECL 26.0) was shown to be methyl 12-acetylmercapto-oleate. Hydrogenolysis and re-methylation gave methyl 12-mercapto-oleate (ECL 23.7) which could be acetylated to the acetylmercapto ester (ECL 26.0). The evidence for these structures is described below.

(i) The infrared spectrum of the acetylmercapto ester contained a strong absorption band due to carbonyl stretching at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum showed a three-proton singlet at 7.72τ ($SCOCH_3$).

(ii) Subjected to Mozingo hydrogenolysis (Raney nickel) the mercapto ester gave methyl stearate (identity on TLC and GLC).

(iii) After von Rudloff oxidation followed by methylation (boron trifluoride/methanol) the oxidation product showed a GLC peak for methyl nonanedioate as the only dibasic ester.

Band B (25-30%) gave no peak on GLC and was probably the dimer of methyl 12-mercapto-oleate. Mozingo hydrogenolysis afforded methyl stearate. On treatment with sodium borohydride band B remained unchanged but reduction with lithium aluminium hydride gave 12-mercapto-octadec-cis-9-enol identical in its IR spectrum (O-H stretching at 3500 cm^{-1}) with that obtained by lithium aluminium hydride reduction of methyl 12-mercapto-oleate. Both samples of the alcohol gave identical bis TMS derivatives [ECL 20.9 (70%) and 19.9 (20%), the latter peak may be that of a mono TMS derivative] and bis TFA derivatives (ECL 20.3). The infrared spectrum of the trifluoroacetylated product showed three strong carbonyl absorption bands at 1780 ($OCOCF_3$), 1735 ($COOCH_3$) and 1700 ($SCOCF_3$) cm^{-1} whilst its NMR spectrum contained characteristic signals at 4.62 (m, 2H, -CH=CH-) and 5.70τ (t, 2H, $-CH_2OCOCF_3$).

Methyl 12-mercapto-oleate was oxidised to its dimer when treated with iodine. The monomeric and dimeric esters gave the same monomeric alcohol when reduced with lithium aluminium hydride (identity on GLC and IR and NMR spectra of the bis TMS and TFA derivatives).

Band C (4%) was probably unreacted mesylate on the basis of its behaviour on TLC and GLC (peaks at 19.6 (major), 20.3, 20.8 and 23.3, thought to be isomeric 18:2 ester formed by decomposition on the column).

Band D (ca 10%) gave no peak on GLC even after methylation (boron trifluoride/methanol) and was not examined further.

1.1d Methyl 12-mercapto-elaidate

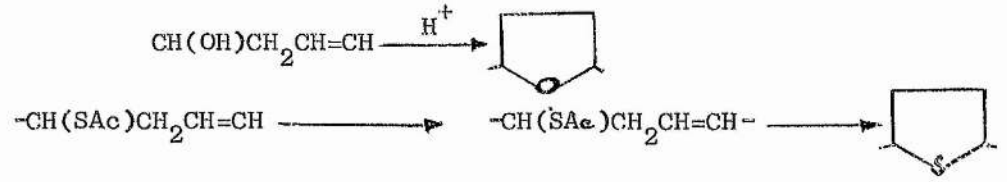
Stereomutation of methyl ricinoleate was accomplished by keeping it with β-mercaptopropionic acid in daylight for 6-7 days[119]. Careful separation by Ag^+TLC afforded methyl ricinelaidate which was converted to its mesyloxy ester in the usual manner.

Methyl 12-mesyloxyelaidate was reacted with sodium hydrogen sulphide in dimethylformamide at room temperature for 30 hours. The product was separated into four fractions of which the least polar (60%) was isolated and showned to be methyl 12-mercapto-elaidate.

On GLC this component gave peaks of ECL 19.6, 20.3, 22.3 and 23.9 (major). After acetylation the main GLC peak was at 26.1 with complete absence of the peak at ECL 23.9. The acetylated product contained characteristic infrared absorptions at 970 ($-CH\overset{t}{=}CH-$), 1685 ($SCOCH_3$) and 1725 ($COOCH_3$) cm^{-1}.

Methyl ricinoleate and several related esters furnished

methyl 9,12-epoxystearate (ca 30%) when treated with p-toluene sulphonic acid in methanol[120].



A comparable reaction was carried out on methyl 12-acetymercapto-elaidate. After refluxing this ester with methanolic sulphuric acid for 2 hours the product was shown to be the dimer of the mercapto ester along with other components on TLC. GLC showed the complete absence of starting material (ECL 26.1) and the presence of 18:2 esters (mainly of ECL 19.6), methyl 12-mercapto-elaidate (23.9, 40%) and an additional component of ECL 24.2 (5%). This may be methyl 9,12-epithiostearate which also has an ECL of 24.2

1.1e Methyl 9,12-epithiostearate

Reaction of methyl 9-mesyloxyoctadec-cis-12-enoate with sodium hydrogen sulphide in dimethylformamide solution at room temperature overnight gave a product which was separated into three fractions (A, B and C) on prep TLC. These were shown to be methyl 9,12-epithiostearate (70-75%), dimer (15-20%) and unreacted starting material (5%)

(R = $CH_3(CH_2)_4$, R' = $(CH_2)_7COOCH_3$

Fraction A (70-75%, ECL 24.2) was considered to be methyl 9,12-epithiostearate on the basis of the following evidence.

(i) On treatment with acetyl chloride and with trifluoroacetic anhydride the product showed no change in its behaviour on TLC and

GLC nor in its IR spectrum

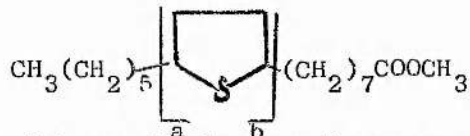
(ii) The NMR spectrum contained a signal at 6.45-6.95τ (m, 2H, -CH(S)CH_2CH_2CH-) and complete absence of any olefinic signal at 4.5τ.

(iii) Unchanged starting material was recovered after an attempted desulphurisation with methyl iodide in refluxing acetone (a characteristic reaction for desulphurisation of 1,2-epithio compounds [121]).

(iv) Subjected to Mozingo hydrogenolysis it gave methyl stearate.

(v) A carefully purified sample of fraction A using double development on prep TLC was analysed. Found: C, 69.35; H, 10.96: calc. $C_{19}H_{36}O_2S$: C, 69.56; H, 10.98%.

(vi) The mass spectrum showed a molecular ion peak at m/e 328 and a base peak at m/e 171 which is a characteristic fragment of methyl 9,12-epithiostearate. Peaks were observed at: 328 (M, 40), 297 (M-31, 27), 243 (a, 57), 211 (a-32, 68), 173 (b+2, 11), 172 (b+1, 25), 171 (b, 100), 87 (c, 96) and members of the series $(CH_2)_nCOOCH_3$ (59-157), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-153).



Fragments a and b result from α-cleavage. Fragment a can further lose 32 (CH_3OH) mass units, presumably from ester function. Fragment c is the central unit remaining after the two α-cleavages a and b.

(vii) Finally the structure was confirmed by comparison with a synthetic sample of methyl 9,12-epithiostearate prepared by a completely independent route (See section 2).

Fraction B (15-20%) had no peak on GLC and may contain disulphides formed during the reaction.

Fraction C (5%) showed the same TLC behaviour as the starting material.

1.1f Methyl 9-mercapto-octadec-cis-12-enoate

As reported in the previous section the major product of the reaction between methyl 9-mesyloxyoctadec-cis-12-enoate and sodium hydrogen sulphide is not the corresponding mercapto ester but methyl 9,12-epithiostearate (70%). When the reaction product was examined immediately after isolation it showed peaks at 19.4 (5%, presumably 18:2), 23.6 (20%), and 24.2 (73%, methyl 9,12-epithiostearate). Two days later only a peak of 24.2 was observed. This also happened when the product was stored in petrol ether solution and in an inert atmosphere.

Immediate acetylation of the quickly isolated crude product gave peaks at 24.2 (45%) and 26.1 (55%) and the product gave four fractions (A-D) on prep TLC.

Band A (35%) showed only one GLC peak (ECL 24.2) after the careful TLC separation (double development). It was considered to be methyl 9,12-epithiostearate since it showed identical behaviour on TLC and GLC with an authentic sample of this epithio ester.

Band B (55%, ECL 26.1). The infrared spectrum of this fraction contained a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum gave an olefinic two-proton signal at 4.60τ and a three-proton singlet at 7.72τ ($SCOCH_3$). On the basis of this evidence it was considered to be methyl 9-acetylmercapto-octadec-cis-12-enoate.

When a sample of this ester was kept at room temperature overnight with a methanolic solution of sulphuric acid, the product gave two peaks of ECL 24.2 (40%) and 26.1 (60%). Only

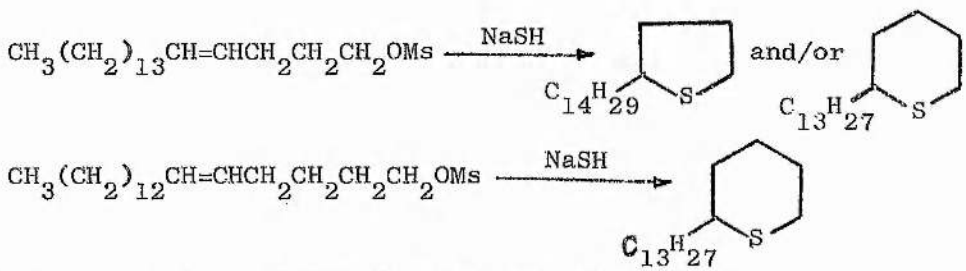
the peak of ECL 24.2 was observed when the acetylmercapto ester was refluxed with methanolic sulphuric acid for 2 hours.

Band C (15%) gave no peak on GLC and was possibly the dimer of methyl 9-mercapto-octadec-cis-12-enoate. On Mozingo hydrogenolysis (Raney nickel) methyl stearate resulted.

Band D (5%) gave no peak on GLC even after methylation (boron trifluoride/methanol) and was not examined further.

1.1g 1-Mercapto-octadec-4- and 5-ene

When methyl 9-mesyloxyoctadec-cis-12-enoate reacted with sodium hydrogen sulphide, the major product (70%) was a cyclic sulphide - methyl 9,12-epithiostearate. The result with this γ-mesyloxy alkene was in contrast to that obtained with a β-mesyloxy alkene (methyl 12-mesyloxy oleate) which gave the corresponding mercapto ester and its dimer and no cyclic sulphide. To extend this observation we examined the reaction of sodium hydrogen sulphide with another γ-mesyloxy alkene (1-mesyloxy-octadec-4-ene) and with a δ-mesyloxy alkene (1-mesyloxyoctadec-5-ene) which might conceivably also give a cyclic sulphide thus:



This possibility was of added interest in that we had not previously obtained a 1,5-epithio derivative.

When 1-mesyloxyoctadec-4-ene was treated with sodium hydrogen sulphide in dimethylformamide solution, the product contained the expected 2-tetradecyltetrahydrothiophen along with

1-mercapto-octadec-4-ene and its dimer but no 2-tridecyltetrahydrothiopyran. In contrast 1-mesyloxyoctadec-5-ene furnished 1-mercapto-octadec-5-ene and its dimer but very little 2-tridecyltetrahydrothiopyran.

These unsaturated mesylates were prepared in the usual manner from the corresponding octadecenoates which were in turn obtained from the synthetic esters[122] by reduction with lithium aluminium hydride

$$RCO_2Me \longrightarrow RCH_2OH \longrightarrow RCH_2OMs$$

Reaction of 1-mesyloxyoctadec-4-ene with sodium hydrogen sulphide

1-Mesyloxyoctadec-4-ene reacted with sodium hydrogen sulphide in dimethylformamide solution and the product was separated into fractions A (43%), B (13%) and C (30%).

Fraction A (43%) which gave no peak on GLC was considered to be the dimer of 1-mercapto-octadec-4-ene. On reduction with lithium aluminium hydride it furnished two subfractions A_1 (50%) and A_2 (20%).

Subfraction A_1 (ECL 17.8) was probably 1-mercapto-octadec-4-ene since it reacted with acetyl chloride to give a product (ECL 21.2) with strong infrared absorption at 1685 cm^{-1} ($SCOCH_3$).

Subfraction A_2 (ECL 18.6) was 2-tetradecyltetrahydrothiophen. It had the same ECL as an authentic sample of this compound and appeared to be unchanged after treatment with acetyl chloride.

Fraction B (13%). This material showed two peaks on GLC [ECL 17.8 (60%) and 18.6 (40%)] but gave single spot on TLC. Its NMR spectrum contained a two-proton olefinic signal at 4.70τ. After reacting with acetyl chloride it gave the corresponding acetylmercapto compound (ECL 21.2) with a strong infrared

absorption band at 1685 cm^{-1} ($SCOCH_3$) and NMR signals at 4.70 (2H, CH=CH) and 7.74τ(3H, $SCOCH_3$). It is concluded that fraction B is mainly 1-mercapto-octadec-4-ene but that this compound was unstable and is partially converted to 2-tetradecyltetrahydrothiophen during GLC.

Fraction C (30%, ECL 18.6) was considered to be 2-tetradecyltetrahydrothiophen. Its NMR spectrum contained a signal at 6.68-6.82 (m, 1H, $-CH_2CH(S)CH_2CH_2CH_2$) and 7.28τ (t, 2H, $-CH_2CH(S)CH_2CH_2CH_2$) with a complete absence of an olefinic signal at 4.7τ. The mass spectrum confirmed the identity with a molecular ion peak at m/e 284 and a base peak at m/e 87 arising from cleavage from the heterocyclic ring. Members of the series C_nH_{2n-1} (55-181) and C_nH_{2n+1} (57-169) were also present.

Reaction of 1-mesyloxyoctadec-5-ene with sodium hydrogen sulphide

1-Mesyloxyoctadec-5-ene and sodium hydrogen sulphide were reacted at room temperature in dimethylformamide solution and the product was separated into three fractions:- A (60%), B (30%) and C (ca 3%).

Fraction A (60%) with no GLC peak was believed to be the dimer of 1-mercapto-octadec-5-ene. Lithium aluminium hydride reduction furnished a product which showed a single spot on GLC. After acetylation its infrared spectrum showed strong absorption band at 1685 ($SCOCH_3$) cm^{-1} whilst its NMR spectrum contained an olefinic signal at 4.70τ and a characteristic three-proton signal at 7.76τ ($SCOCH_3$).

Fraction B (30%) with two GLC peaks of ECL 17.8 (90%) and 18.1 (10%) was considered to be 1-mercapto-octadec-5-ene. It contained

an olefinic signal at 4.70τ in its NMR spectrum. After the reaction with acetyl chloride the product had a single peak on GLC (21.2), showed strong infrared absorption at 1685 ($SCOCH_3$) cm^{-1}, and gave signals at 4.70 (-CH=CH-) and at 7.76τ ($SCOCH_3$) in its NMR spectrum.

The peak of ECL (18.6, 10%) was possibly of 2-tridecyl-tetrahydrothiopyran formed during GLC.

Fraction C (ca 3%, ECL 18.1) may be 2-tridecyltetrahydrothio-pyran but further examination was not possible due to lack of material.

1.2 Reaction of mesyloxy esters with potassium thiolacetate

As an alternative to the reaction of mesyloxy compounds with sodium hydrogen sulphide we have found their reaction with excess of potassium thiolacetate to be useful. Reaction occurs in acetone (reflux for 7 hr) or in dimethylformamide (100° for 2 hr) and we prefer the shorter reaction, the product is an acetyl-mercapto compound which can be hydrolysed in acidic or alkaline solution.

1.2a Methyl 12-mercaptostearate

Methyl 12-mesyloxystearate was refluxed with potassium thiolacetate in dry acetone for $7\frac{1}{2}$ hours. Prep TLC of the product afforded a single component which showed only one peak (ECL 25.4) on GLC. This was considered to be methyl 12-acetylmercapto-stearate. Its infrared spectrum contained a strong absorption band

at 1685 cm^{-1} ($SCOCH_3$) whilst the NMR spectrum showed signals at 6.38-6.42 (m, 1H, -CHSCOCH$_3$) and at 7.72τ (s, 3H, -CHSCOCH$_3$).

Hydrolysis (methanolic sulphuric acid) of the acetylmercapto ester furnished methyl 12-mercaptostearate (ECL 23.2) as the major product along with some dimer formed by the oxidation during reaction. The TFA-derivative (ECL 21.5) of the mercapto ester showed the expected infrared absorption band at 1700 ($SOCOCF_3$) cm^{-1}.

1.2b Methyl 12-mercapto-oleate

Reaction of methyl 12-mesyloxyoleate with potassium thiol-acetate in dimethylformamide at 100°C for 3 hours furnished methyl 12-acetylmercapto-oleate (70%, ECL 26.0). Its infrared spectrum gave a strong diagnostic absorption band at 1685 ($SCOCH_3$) cm^{-1} whilst its NMR spectrum contained a two-proton olefinic signal at 4.62 and a three-proton singlet at 7.72τ ($SCOCH_3$).

Hydrolysis (methanolic sulphuric acid) of methyl acetyl-mercapto-oleate afforded methyl 12-mercapto-oleate (ECL 23.7).

1.2c Methyl 12-mercapto-elaidate

Methyl 12-mesyloxyoctadec-trans-9-enoate and potassium thiol-acetate reacted in a similar way to give methyl 12-acetylmercapto-elaidate (75%, ECL 26.1). Its infrared spectrum contained characteristic absorption bands at 970 (-CH=^{t}CH-), 1685 ($SCOCH_3$) and 1735 ($COOCH_3$) cm^{-1}.

When a sample of methyl 12-mercapto-elaidate (ECL 26.1) was refluxed with a methanolic solution of sulphuric acid for 2 hours, the product gave two peaks of ECL 23.9 (90%) and 24.2 (5%). TLC showed also the presence of some dimer.

In contrast methyl 12-mercapto-oleate gave only one peak of ECL 23.7 in addition to dimer. The additional peak of ECL 24.2 is

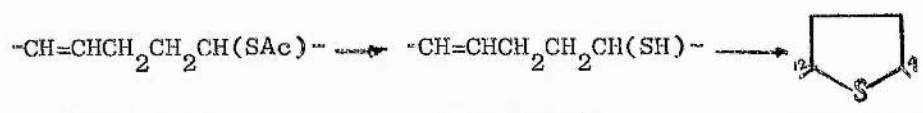
probably methyl 9,12-epithiostearate which also has an ECL of 24.2

1.2d Methyl 9-acetylmercapto-octadec-cis-12-enoate

Reaction of methyl 9-mesyloxyoctadec-cis-12-enoate and potassium thiolacetate furnished methyl 9-acetylmercapto-octadec-cis-12-enoate (50%, ECL 26.1). Its infrared spectrum showed an absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum contained a two-proton olefinic signal at 4.7 and a three-proton singlet at 7.74 τ ($SCOCH_3$). When the acetylmercapto ester was kept at room temperature with methanolic sulphuric acid the product showed its original peak (26.1, 60%) and a new peak (24.2, 40%). When refluxed for 2 hours the original peak disappeared completely and only the new peak (24.2) was observed.

The hydrolysis was repeated in the presence of Zn/Hg and degassed water was used during the recovery of the product in an attempt to obtain mercapto alkenoate ester without further reaction. The product then gave a single spot on TLC but showed two peaks on GLC at 23.6 (20%) and 24.2 (75%). Its NMR spectrum contained a two-proton olefinic signal at 4.72τ.

It is concluded that methyl 9-mercapto-octadec-cis-12-enoate is an unstable compound readily converted to a cyclic sulphide and that this reaction may also occur during gas liquid chromatography.



(ECL 26.1) (ECL 23.6) (ECL 24.2)

1.2e 1-Mercapto-octadec-4- and 5-ene

(i) 1-Acetylmercapto-octadec-4-ene

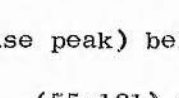
1-Mesyloxyoctadec-4-ene and potassium thiolacetate were

heated at 100°C for 3 hours. Prep TLC of the product afforded 1-acetylmercapto-octadec-4-ene (85%, ECL 21.2). Its infrared spectrum showed strong absorption band at 1685 ($SCOCH_3$) cm^{-1} and its NMR spectrum contained an olefinic signal at 4.70 and a characteristic three-proton signal at 7.74τ ($SCOCH_3$).

After refluxing with methanolic sulphuric acid for 3 hours 1-acetylmercapto-octadec-4-ene gave a product which showed two bands, A (70%) and B (25%) on prep TLC.

Band A [70%, ECL 17.8 (60%) and 18.6*(40%)] was believed to be 1-mercapto-octadec-4-ene since it reacted with acetyl chloride to give 1-acetylmercapto-octadec-4-ene which showed strong infrared absorption at 1685 ($SCOCH_3$) cm^{-1} and a characteristic NMR signal at 7.74τ (s, 3H, $SCOCH_3$).

Band B (25%, ECL 18.6) was probably 2-tetradecyltetrahydrothiophen. Its NMR spectrum showed a broad signal at 6.68-6.82τ (m, 1H, $-CH_2CH(S)CH_2CH_2CH_2$) and 7.28τ (t, 2H, $-CH_2CH(S)CH_2CH_2CH_2$) with a complete absence of olefinic signal at ~4.70τ.

The identity of this component was confirmed by its mass spectrum which showed a molecular ion peak at m/e 284, a characteristic fragment of m/e 87 (base peak) believed to be  , and members of the series C_nH_{2n-1} (55-181) and C_nH_{2n+1} (57-169).

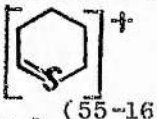
(ii) 1-Acetylmercapto-octadec-5-ene

Reaction of 1-mesyloxyoctadec-5-ene and potassium thiolacetate in dimethylformamide solution at 100°C for 3 hours afforded 1-acetylmercapto-octadec-5-ene (80%, ECL 21.2). Its infrared spectrum showed strong absorption at 1685 ($SCOCH_3$) cm^{-1} whilst its NMR

* This peak was possibly formed during GLC for the reason already described.

spectrum contained a two-proton signal at 7.74τ ($SCOCH_2$).

When 1-acetylmercapto-octadec-5-ene was refluxed with methanolic sulphuric acid for 3 hours under nitrogen the isolated product showed a single spot on TLC and a single peak on GLC (ECL 17.8). This single component was believed to be 1-mercapto-octadec-5-ene since it reacted with acetyl chloride to furnish 1-acetyl-mercapto-octadec-5-ene [ECL 21.2, IR absorption at 1685 cm^{-1} ($SCOCH_3$) and NMR signal at 7.75τ (s, 3H, $SCOCH_3$)].

When 1-mercapto-octadec-5-ene was kept over an ethanolic solution of iodine for 40 hours the product showed a new polar spot (20%, ECL 18.1) along with unreacted starting material. This was considered to be 2-tridecyltetrahydrothiopyran. Its NMR spectrum contained a signal at 7.38-7.60 (m, 1H, $-CH(S)CH_2CH_2CH_2CH_2$) and 8.81τ (t, 2H, $-CH(S)CH_2CH_2CH_2CH_2$) whilst its mass spectrum showed a molecular ion peak at m/e 284, a base peak of m/e 101 believed to be  and members of the series C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).

When 1-mercapto-octadec-4-ene and 5-ene in ether solutions were left in air for one week, they each furnished two products with the complete disappearance of starting material. Over shorter periods of time TLC showed the slow formation of the appropriate dimer and of a second component of ECL 18.6 (2-tetradecyltetrahydrothiophen) from the 4-ene and 18.1 (2-tridecyltetrahydrothiopyran) from the 5-ene.

1.3 Free radical addition of thiolacetic acid to alkenoic acids or esters

The free radical addition of thiolacetic acid to olefins was first reported by Holmberg[123] and by Ipatieff and Friedmann[124].

Since then similar additions to a large variety of olefins have been reported[31,126-128]. Swern et al[32,129-133] described the addition of thiolacetic acid to long-chain monounsaturated compounds using ultra-violet light as in the initiator. Addition of mercaptans to olefins is thus a well known reaction and generally follows a free-radical mechanism[134-141].

$$RCH=CHR' + CH_3COSH \longrightarrow RCH(SAc)CH_2R' + RCH_2CH(SAc)R'$$

Addition of thiolacetic acid to oleic acid, methyl oleate, methyl 12-hydroxyoleate and methyl 9-hydroxy-octadec-cis-12-enoate was of interest in connection with the preparation of thiols.

In the present work addition was initiated by peroxides at 60-70° in an inert atmosphere. Mixed acetyl mercapto compounds were obtained in excellent yield and were hydrolysed with aqueous alcoholic alkali or methanolic sodium methoxide.

1.3a Methyl 9(10)-mercaptostearate from oleic acid

Pure oleic acid (obtained from olive oil by urea fractionation), thiolacetic acid and ditertiary-butyl peroxide were heated under nitrogen at 60-70° for 18 hours and for another 9 hours after the addition of more ditertiary-butylperoxide. The crude product showed strong absorption bands at 1680 ($SCOCH_3$) and 1700 (COOH) cm^{-1}. Deacetylation (potassium hydroxide in methanol) followed by esterification (boron-trifluoride/methanol) furnished a product which showed a less polar major band along with some polar diffuse bands on prep TLC. Only the major band was isolated and studied in greater detail.

The major band (53%) gave several small peaks on GLC with a major peak of ECL 23.3 (80%). After acetylation and purification

by prep TLC using double development methyl 9(10)-acetylmercapto-stearate (ECL 26.0) was isolated. Its infrared spectrum contained a characteristic absorption band at 1685 ($SCOCH_3$) cm^{-1} whilst its NMR spectrum showed a three-proton singlet at 7.72τ ($SCOCH_3$) and a complete absence of any olefinic signal around 4.5τ.

Deacetylation (methanolic sodium methoxide) afforded pure methyl 9(10)-mercaptostearate which showed a single GLC peak of ECL 23.3. Mozingo hydrogenolysis (Raney nickel) on this product furnished methyl stearate (identity on TLC and GLC).

1.3b Methyl 9(10)-mercaptostearate from methyl oleate

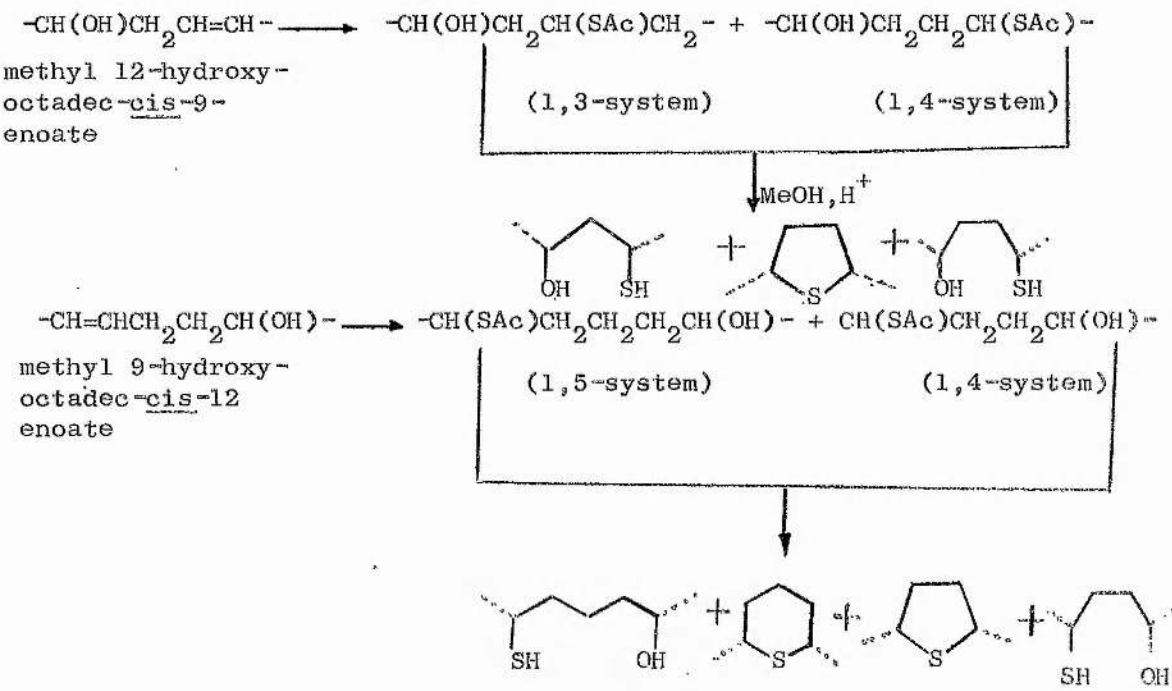
Methyl oleate, thiolacetic acid and ditertiary-butyl peroxide were reacted in the usual manner and the major product (45%, ECL 26.0), isolated by prep TLC, was shown to be methyl 9(10)-acetylmercaptostearate. Deacetylation (methanolic potassium hydroxide) and then esterification (boron trifluoride/methanol) furnished methyl 9(10)-mercaptostearate (57%, ECL 23.3) along with some dimer (19%). These conclusions are based on the following evidence: (i) After acetylation this component (ECL 26.0) showed a strong infrared absorption band at 1685 cm^{-1} ($SCOCH_3$) whilts its NMR spectrum contained a characteristic three-proton signal at 7.72τ ($SCOCH_3$); (ii) the NMR spectrum of the unacetylated product showed no olefinic signals ($\sim$4.5τ); and (iii) it furnished methylstearate when submitted to Mozingo hydrogenolysis (Raney nickel).

The alleged dimer (19%) gave no peak on GLC, appeared to be unchanged after treatment with sodium borohydride, but furnished methyl stearate on Mozingo hydrogenolysis.

Gunstone and Abbot[142] reported that 1,4-diols cyclise with greater ease in the presence of acid to furnish 1,4-epoxides

and it was therefore of interest to investigate the reaction of compounds containing 1,4-hydroxy-mercapto or 1,4-dimercapto systems. Such compounds are present in the isomeric mixtures of mixtures of methyl 12-hydroxy-9(10)-mercaptostearate and methyl 9-hydroxy-12(13)-mercaptostearate which are readily prepared from methyl ricinoleate and methyl 9-hydroxyoctadec-cis-12-enoate respectively by the free-radical addition of thiolacetic acid.

The acetylmercapto-hydroxy ester containing 1,4- and 1,5-system could conceivably cyclise to yield 1,4-epithio and 1,5-epithio compounds in addition to or as an alternative to O-hetero-cyclic product



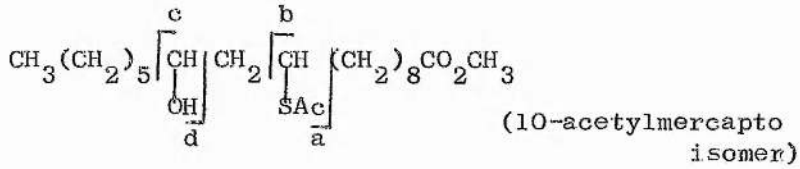
In our study methyl 9(10)-acetylmercapto-12-hydroxystearate and methyl 12(13)-acetylmercapto-9-hydroxystearate were treated with methanolic sulphuric acid. We found that both methyl 12(13)-acetylmercapto-9-hydroxystearate and 9(10)-acetylmercapto-12-hydroxystearate under acidic conditions furnish the methyl 9,12-epithiostearate (20-25%), hydroxymercaptostearate (presumably

mostly 9,13- and 12,10- 50-60%) and some unidentified product (15%). There was no evidence that any methyl 9,13-epithiostearate was obtained.

1.3c Methyl 9(10)-acetylmercapto-12-hydroxystearate

Methyl ricinoleate, thiolacetic acid and ditertiary butyl peroxide were reacted in the usual manner. The major component (61%) showed infrared absorption bands at 3500 (OH), 1735 ($COOCH_3$) and 1685 ($SCOCH_3$) cm^{-1} and its NMR spectrum contained a characteristic three-proton signal at 7.72τ ($SCOCH_3$). The acetylmercapto-hydroxy ester after deacetylation (methanolic sodium methoxide) and re-acetylation (acetic anhydride/anhydrous sodium acetate) gave an O-acetyl S-acetyl compound whose NMR spectrum showed two characteristic three-proton signals at 7.72τ ($SCOCH_3$) and at 8.04τ ($OCOCH_3$).

The mass spectrum of methyl 9(10)-acetylmercapto-12-hydroxystearate showed major peaks at 389 (M+1, 25), 371 (389-18, 47), 329 (389-60, 19), 328 (389-61 or M-60, 9), 327 (M-61, 42), 312 (M-76, 9), 311 (?, 38), 297 (371-74, 9), 295 (371-76 or C-18, 10), 243 (C-60, 25), 115 (d, 7), 97 (d-18, 25). Peaks at 185 (b-74, 4), 163 (b-106, 5), 143 (a-74, 14) probably arise from the 10-acetylmercapto ester and peaks at 171 (b-74 and or a-60, 15), 139 (b-106 and or a-92, 3) from the 9-acetylmercapto ester. The base peak was at 55.



(10-acetylmercapto isomer)

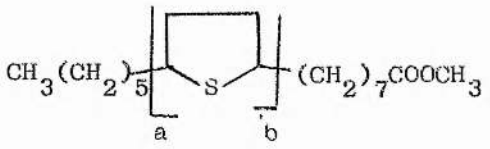
The α-cleavages shown above produce fragment ions. The molecular ion and these fragment ions, as appropriate, lose 18 (H_2O), 31 or

32 (CH_3O or CH_3OH), 42 or 43 (CH_2CO or CH_3CO), and 74 or 75 or 76 (CH_2COS, or CH_3COS or CH_3COSH) mass units.

When methyl 9(10)-acetylmercapto-12-hydroxystearate was refluxed with methanolic sulphuric acid, the product was a mixture of three components A (20-25%), B (50-60%) and C (15%) which were separated by prep TLC.

Band A (20-25%) gave an ECL of 24.2 with an inflection at 24.1. Its NMR spectrum showed a broad signal at 6.50-6.95τ (2H) but signals for $SCOCH_3$ and olefinic protons were absent. This fraction was finally shown to be methyl 9,12-epithiostearate from its mass spectrum which contained a molecular ion peak at m/e 328 and had its base peak at 171. The details of the fragmentation pattern. are discussed below.

Peaks at 328 (M, 28), 297 (M-31, 17), 243 (a, 40), 211 (a-32, 50), 173 (b+2, 7), 172 (b+1, 14), 171 (b, 100), 87 (c, 70) and also peaks of the series $(CH_2)_nCOOCH_3$ (59-143), C_nH_{2n+1} (57-169), and C_nH_{2n-1} (55-153).



Fragments a and b arise from α-cleavage and c is the central unit remaining after two α-cleavages.

Band B (50-60%) showed strong infrared absorption band at 3500 (OH) cm^{-1} and was considered to be methyl 12-hydroxy-9(10)-mercaptostearate.

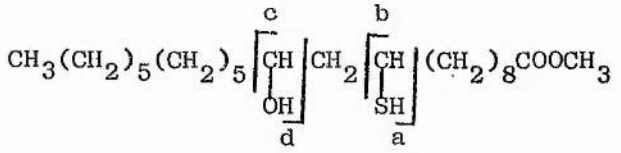
Its bis-trifluoroacetyl derivative (ECL 22.7, 23.0 and 23.5) contained absorption bands at 1700 ($SCOCH_3$), 1735 ($COOCH_3$) and 1770 cm^{-1} ($OCOCF_3$). Deacetylation (methanolic sodium methoxide) and subsequent reaction with trifluoroacetic anhydride regenerated the bis-TFA with no evidence of any cyclised

material. The mass spectrum of band B contained the following peaks:

Peaks at 328 (M-18, 7), 297 (328-31, 5), 243 (c-18, 13), 211 (c-50, 11), 143 (? , 30), 115 (d, 8), 97 (d-18, 15), along with those from C_nH_{2n-3} (67-109), C_nH_{2n-1} (55-101) and $(CH_2)_nCOOCH_3$ (59-125). The base peak is at m/e 55.

Peaks from the 10-mercapto isomer at 183 (b-34, 5), 157 (a-18,1), 151 (b-66, 6) and 123 (a-52, 4).

Peaks from the 9-mercapto isomer at 171 (b-32, and/or a-18, 42), 169 (b-34, 3) and 137 (b-66 and/or a-52, 5).



Fragment ions result from the α-cleavages a-d. The molecular ion and these fragment ions (as appropriate) lose 18 (H_2O), 31 or 32 (CH_3O or CH_3OH), and 33 or 34 (HS or H_2S) mass units.

Band C (15%) gave no peak on GLC even after methylation (boron trifluoride/methanol) or trifluoroacetylation and was not examined further.

1.3d Methyl 12(13)-acetylmercapto-9-hydroxystearate

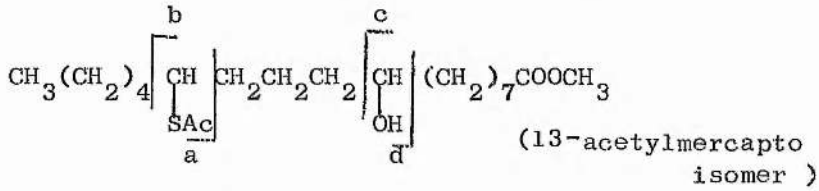
Methyl 9-hydroxyoctadec-cis-12-enoate, thiolacetic acid and ditertiarybutyl peroxide were treated as described previously. The less polar major band (62%) was of methyl 12(13)-acetyl-mercapto-9-hydroxystearate which showed characteristic absorption bands at 1685 ($SCOCH_3$), 1735 ($COOCH_3$) and 3500 (OH) cm^{-1} in its infrared spectrum whilst its NMR spectrum contained a diagnostic three-proton signal at 7.72τ ($SCOCH_3$) and complete absence of olefinic signals at ca 4.5τ. Its mass spectrum contained the

following peaks:

Peaks at 389 (M+1, 11), 371 (389-18, 30), 329 (389-60, 24), 328 (389-61 and/or M-60, 20), 327 (M-61, 52), 312 (M-76, 10), 311 (?, 44), 297 (371-74, 22), 295 (371-76, 18), 213 (d-18, 8), 200 (?, 18), 187 (c, 12), 185 (?, 4), 171 (d-60, 80), 155 (c-32, 30), 143 (?, 22), 137 (c-50, 8), and the base peak at 55.

Peak from the 13-acetylmercapto isomer at 257 (b-60, 5), 243* (b-74, 6), 225 (b-92, 5), 159 (a,6), and 117 (a-42, 8).

Peaks from the 12-acetylmercapto isomer at 243* (b-60, 6), 229 (b-74, 1), 211 (b-92, 1), 173 (a, 8), 131 (a-42, 14).



(13-acetylmercapto isomer)

Fragment ions result from the α-cleavages a-d. The molecular ion and these fragment ions (as appropriate) lose 18 (H_2O), 31 or 32 (CH_3O or CH_3OH), 42 or 43 (CH_2CO or CH_3CO) and 74 or 75 or 76 (CH_2COS or CH_3COS or CH_3COSH) mass units.

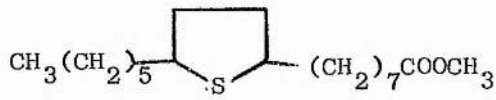
Hydrolysis (methanolic sodium methoxide) of the methyl 12(13)-acetylmercapto-9-hydroxystearate afforded the hydroxy-mercapto ester. Its infrared spectrum gave a strong absorption band at 3500 (OH) cm^{-1}. The infrared spectrum of the bisacetyl and the bis TFA derivative (ECL 22.7, 23.0 and 23.3) showed characteristic bands due to carbonyl stretching at 1685 ($SCOCH_3$), 1700 ($SCOCF_3$) and 1770 ($OCOCF_3$) cm^{-1} respectively whilst the NMR spectrum of the acetylated product contained two diagnostic three-proton signals at 7.72τ ($SCOCH_3$) and 8.05τ ($OCOCH_3$).

* Peaks which may arise from either isomer

Methyl 12(13)-acetylmercapto-9-hydroxystearate was refluxed with methanolic sulphuric acid for 2 hours and the product was separated into three bands A (20-25%), B (50-60%) and C (16%) on prep TLC.

Band A (20-25%, ECL 24.2 with an inflection at 24.1) contained a broad signal at 6.50-6.95τ (2H), and complete absence of olefinic and $SCOCH_3$ signals. Hydrogenolysis of band A furnished methyl stearate. This is considered to be methyl 9,12-epithiostearate on the basis of its identical GLC behaviour with authentic material and of the mass spectrum which contained a molecular ion peak at m/e 328 and a characteristic peak at 171. Other major peaks are detailed below.

Peaks at 328 (M, 28), 297 (M-31, 16), 243 (a, 38), 211 (a-32, 30), 173 (b+2, 8), 172 (b+1, 13), 171 (b, 100), 87 (c, 50) and



peaks of the series $(CH_2)_nCOOCH_3$ (59-143), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-153). Fragments and and b result from α-cleavage and c is the central unit remaining after two α-cleavages.

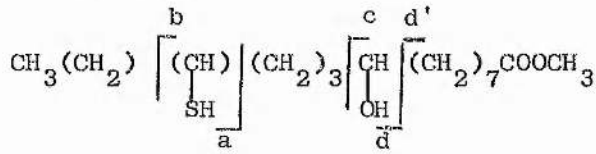
Band B (50-60%) was considered to be methyl 9-hydroxy-12(13)-mercaptostearate. Its infrared spectrum showed a strong absorption band at 3500 (OH) cm^{-1}. After acetylation band B contained a strong absorption band at 1685 ($SCOCH_3$) in its infrared spectrum whilst its NMR spectrum gave two three-proton signals at 7.72τ ($SCOCH_3$) and 8.04τ ($OCOCH_3$). Band B after trifluoroacetylation (ECL 22.7, 23.0, and 23.3) showed infrared absorption bands at 1700 ($SCOCF_3$) and 1770 ($OCOCF_3$) cm^{-1}. Mass spectrum of band B

contained the following peaks.

Peaks at 328 (M-18, 21), 297 (328-31, 15), 295 (328-33, 15), 263 (297-34 and/or 295-32, 12), 213 (?, 12), 189 (d, 5), 187 (c, 9), 185 (? , 15), 171 (d-18, 57), 169 (c-18, 5), 157 (d', 33), 155 (c-32 and/or d-34, 51), 153 (?, 18), 143 (?, 15), 138 (?, 15), 137 (c-50 and/or d-52, 15), 135 (?, 11) along with those from C_nH_{2n-3} (67-109), C_nH_{2n-1} (55-111) and $(CH_2)_nCOOCH_3$ (59-129) and the base peak at 55.

Peaks from 13-mercapto isomer at 257 (b-18, 18), 243* (b-32, 15), 225 (b-50, 39), 117 (a,9), and 83* (a-34, 75).

Peaks from 12-mercapto isomer at 243* (b-18, 15), 211 (b-50, 15), 131 (a, 9) and 97* (a-34, 57).



The α-cleavages shown above produce fragment ions. The molecular ion and the fragment ions as appropriate, lose 18 (H_2O), 31 or 32 (CH_3O or CH_3OH) and 33 or 34 (HS or H_2S) mass units.

Band C (16%) with no GLC peak even after methylation (boron trifluoride/methanol) or trifluoroacetylation was not examined further.

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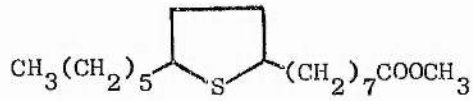
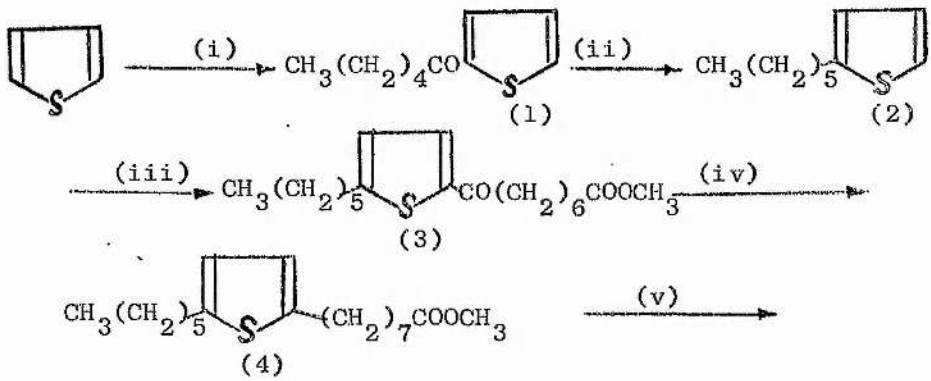
* Peaks which may arise from either isomer

2. Synthesis of methyl 9,12-epithiostearate

Chromatographic, spectroscopic and chemical evidence indicated that the major product (70%) of the reaction between methyl 9-mesyloxyoctadec-cis-12-enoate and sodium hydrogen sulphide was methyl 9,12-epithiostearate.



This structure was finally confirmed by comparison with a synthetic sample prepared from thiophen by the sequence of reactions outlined below



(i) $CH_3(CH_2)_4COCl;SnCl_4$ (ii) $N_2H_4;(HOCH_2CH_2)_2O,KOH$

(iii) $ClCO(CH_2)_6COOCH_3;SnCl_4$ (iv) $N_2H_4;(HOCH_2CH_2)_2O,KOH$

(v) Pd/H_2

The use of thiophen as a chain-extender is well known[143-148]. After acetylation or alkylation of thiophen, at positions 2 and 5, the disubstituted derivative is usually submitted to hydrogenolysis to give a saturated sulphur-free acid. By adaptation of the last stage we converted the thiophen to a tetrahydrothiophen derivative.

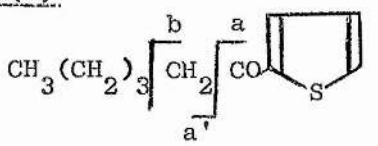
Thiophen was acylated with hexanoyl chloride in benzene in the presence of stannic chloride to furnish 2-hexanoylthiophen (1) which was converted into 2-hexylthiophen (2) by Wolff-Kishner reduction[149,150]. Acylation of 2-hexylthiophen with the C_8 half-ester acid chloride furnished methyl 8-(2',5'-hexylthienyl)-8-oxo-octanoate (3) which was reduced to methyl 8-(2',5'-hexylthienyl)octanoate (4).

Difficulties were encountered in attempts to reduce methyl 8-(2',5'-hexylthienyl)octanoate to methyl 9,12-epithiostearate. In the past, reduction of such sulphur containing compounds has been accomplished by chemical reduction rather than catalytic hydrogenation because of the poisoning effect of reduced sulphur on the catalysts[5]. However, the catalytic hydrogenation of thiophen has been reported[151-153]. The thiophen derivative (4) was hydrogenated by shaking with hydrogen at atmospheric pressure using 10% palladium on charcoal as catalyst in the presence of a few drops of sulphuric acid[29]. Complete hydrogenation required 3-4 days with addition of more catalyst at 24 hour intervals and furnished methyl 9,12-epithiostearate (50%), methyl stearate (30%), and an unidentified product (10%).

The synthetic methyl 9,12-epithiostearate (5) showed identical TLC and GLC behaviour (ECL 24.2) to that of the major component isolated from the methyl 9-mesyloxyoctadec-cis-12-enoate and sodium hydrogen sulphide reaction. It also exhibited similar NMR signals at 6.40 (s, 3H, $-COOCH_3$), 6.45-6.95 (br.s, 2H, $-CH(S)CH_2CH_2CH-$), 7.82 (t, 2H, $-CH_2COOCH_3$), 8.65 (br.s, 26H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-). Its mass spectrum, detailed below along with those of the intermediate products of this synthetic sequence, is also identical to that

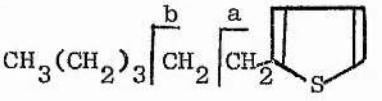
of the epithiostearate obtained previously.

2-Hexanoylthiophen (1)



Major peaks: 182 (M, 6), 126 (b+1, 10), 111 (a, 12), 71 (a', 90). Fragments a and a' result from α-cleavage and b from β-cleavage accompanied by McClafferty rearrangement.

2-Hexylthiophen (2)

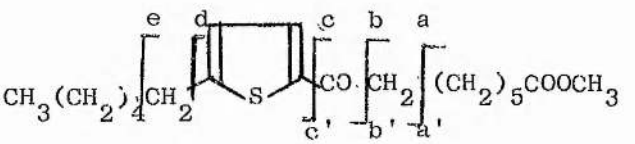


Major peaks: 168 (M, 27), 153 (?, 7), 111 (b, 7), 99 (a+2, 11), 98 (a+1, 50) and 97 (a, 100).

Fragments a and b result from α and β-cleavage respectively.

Methyl 8-(2',5'-hexylthienyl)-8-oxo-octanoate (3)

Major peaks: 338 (M, 28), 306 (M-32, 20), 267 (e, 4), 253 (d, 20), 223 (?, 24), 211 (a'+2, 48), 210 (a'+1, 100), 198 (?, 18), 197 (b'+2, 32), 196 (b'+1, 95), 157 (c', 6), 140 (f+2, 18), 139 (c-32 and/or f+1, 94), 138 (f, 28), 126 (g+2, 6), 125 (g+1, 14), 124 (g, 15), 111 (b-32, 30), 97 (a-32, 42), 96 (?, 22) and also at 83, 74, 69, 67, 59, 57 and 55.

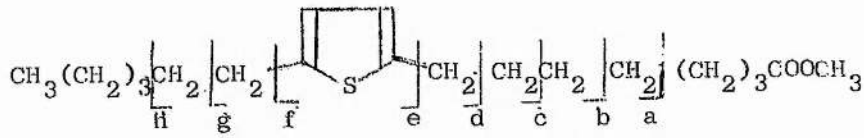


Fragments a-e result from the cleavage shown. Fragments a, b and c lose a further 32 mass units (CH_3OH), presumably from the ester function. Fragment f is the central unit remaining after β-cleavage, as in a and e and fragment g is another central unit

remaining after two α-cleavages either d and c or b and d.

Methyl 8-(2',5'-hexylthienyl)octanoate (4)

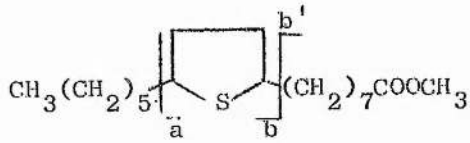
Major peaks: 325 (M+1, 80), 324 (M, 94), 294 (325-31, 22), 293 (M-31, 96), 267 (h, 20), 264 (?, 18), 255 (g+2, 20), 254 (g+1, 58), 253 (g, 96), 239 (f, 28), 223 (a, 20), 221 (g-32, 20), 209 (b, 20), 207 (f-32, 28), 197 (c+2, 18), 196 (c+1, 14), 195 (c, 76), 183 (d+2, 52), 182 (d+1, 96), 181 (d, 100), 169 (c+2, 24), 168 (e+1, 26), 167 (e, 96) and also at 151, 142, 137, 125, 124, 123, 113, 112, 111, 110, 99, 98, 97, 95, 87, 83, 81, 79, 77, 74, 69, 67, 57 and 55.



Fragments f, g and h lose a further 32 mass units presumably from the ester function.

Methyl 9,12-epithiostearate (5)

Major peaks: 328 (M, 27), 297 (M-31, 16), 243 (a, 38), 211 (a-32, 50), 173 (b+2, 15), 172 (b+1, 17), 171 (b, 100), 158 (b+1, 40), 157 (b', 10), 87 (c, 97), and also members of the series $(CH_2)_nCOOCH_3$ (59-143), C_nH_{2n+1} (57-141) and C_nH_{2n-1} (55-139).



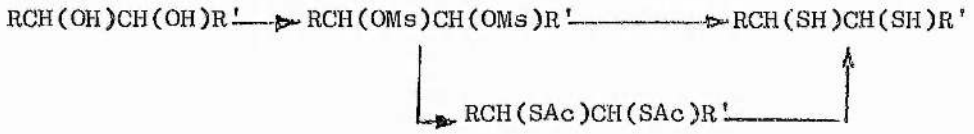
Fragments a and b result from α-cleavage. Fragments a and b' can lose 32 mass units from the ester function. The ion c is the central unit remaining after two α-cleavages a and b.

3. Preparation of dimercapto C_{18} esters and related cyclic sulphides

3.1 Reaction of vicinal dimesyloxy esters with sodium hydrogen sulphide

The preparation of vicinal dimercapto esters from the corresponding dihydroxy esters via the dihalides by the direct interaction of alkali-metal hydrogen sulphide[54-57] or thiolacetate[55,58] has been reported. Chapman and Owen claim that the conversion of dihydroxy compounds to the corresponding mesyloxy and tosyloxy derivatives followed by their reaction with potassium thiolacetate and subsequent hydrolysis of the acetyl-mercapto compound is a more suitable route to dimercapto compounds.

We have prepared vicinal dimercapto esters from the corresponding dimesyloxy esters (erythro and threo) by reaction with sodium hydrogen sulphide at room temperature for 24-30 hours or with potassium thiolacetate at 100°C for 4 hours thus:



Reaction of vicinal dimesyloxy esters with sodium hydrogen sulphide afforded the desired dimercapto ester along with some dimer and some unidentified polar material. The reaction with potassium thiolacetate yielded the acetylmercapto esters only.

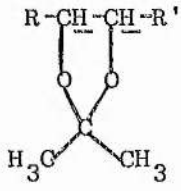
The identity of the vicinal dimercapto esters was confirmed by the combined study of the IR, NMR and mass spectra of these compounds and of their diacetyl and isopropylidene derivatives.

It is considered that the erythro and threo dimesyloxy esters furnished threo and erythro-dimercaptans respectively when treated with sodium hydrogen sulphide. This conclusion

arose from a comparison of the isopropylidene derivative of the vicinal dimercaptans with known information about the isopropylidene derivative of the related dihydroxy compounds. The preparation and properties of these O-analogues[155,156] is described first.

3.1a Methyl erythro- and threo-9,10-isopropylidenedioxystearate

For the purpose of comparison with the corresponding sulphur compounds the isopropylidene derivatives of methyl erythro- and threo-9,10-dihydroxystearate were prepared and examined by NMR and mass spectrometry.

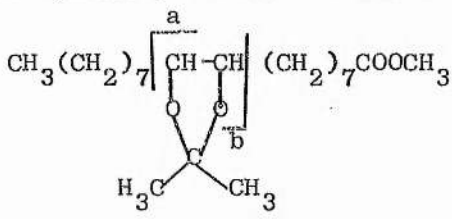


Ia R = $CH_3(CH_2)_7$ I& R'=$(CH_2)_7COOCH_3$

Ib R = R' = CH_3

The two hydrogens indicated in structure (Ia) gave an NMR signal at 6.06-6.20τ in the cis isomer (from the erythro diol) and at 6.54-6.66τ in the trans isomer (from the threo diol) and this observation is similar to that reported by Ewing[157] and by Anet[158] for the simple cis (5.85τ) and trans (6.58τ) compounds (Ib).

Both isopropylidene esters (Ia) gave peaks at 355 (M-15) in their mass spectra. Other details are given below.

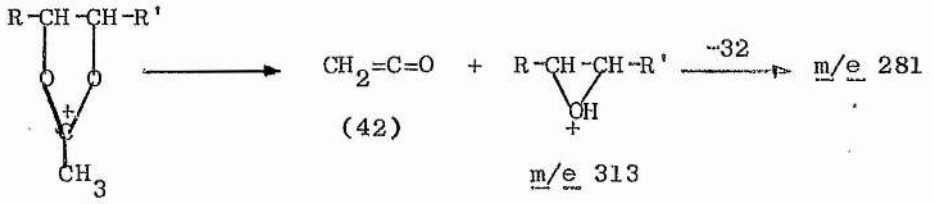


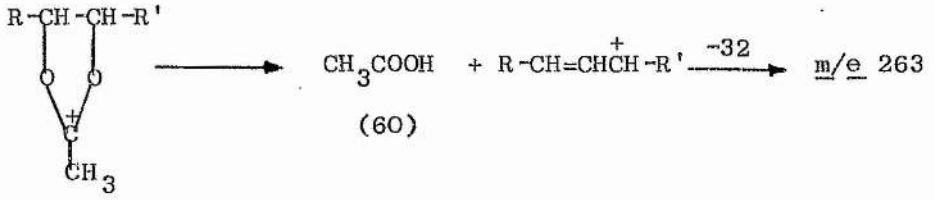
Major peaks in the cis isomer: 355 (M-15, 100), 281 (355-74, 13),

263 (355-92, 14), 257 (a, 1), 213 (b, 6), and members of the series $(CH_2)_nCOOCH_3$ (59-171), C_nH_{2n+1} (57-169), and C_nH_{2n-1} (55-153).

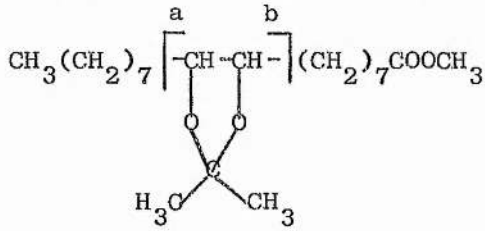
Major peaks in the trans isomer: 355 (M-15, 100), 281 (M-74, 26), 263 (355-92, 5), 257 (a, 1), 213 (b, 6) and members of the series $(CH_2)_nCOOCH_3$ (59-171), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-153).

The compound does not show a molecular ion peak but the molecular weight can be derived from the existence of the base peak at m/e 355 (M-15) resulting from loss of a methyl group from the dioxalane ring. The peaks at m/e 281 and at 263 have been explained[159] in terms of loss of ketene and acetic acid respectively accompanied in each case by the further loss of methanol (32).





The low intensity fragments at 257 and 213 resulting from α-cleavage are of considerable structural significance since



they indicate the position of the ring.

3.1b Methyl threo-9,10-dimercaptostearate from methyl erythro-9,10-dihydroxystearate

The product of the reaction between methyl erythro-9,10-dimesyloxystearate and sodium hydrogen sulphide was separated into two fractions A (50%) and B (20%) by prep TLC.

Band A (50%) which showed a late-running peak of ECL ~32 on GLC was believed to be methyl threo-9,10-dimercaptostearate. The infrared spectrum showed a characteristic absorption band at 2580 (SH) cm^{-1} whilst its NMR spectrum contained a two-proton signal at 7.16τ (-CH(SH)CH(SH)-) in addition to other usual signals.

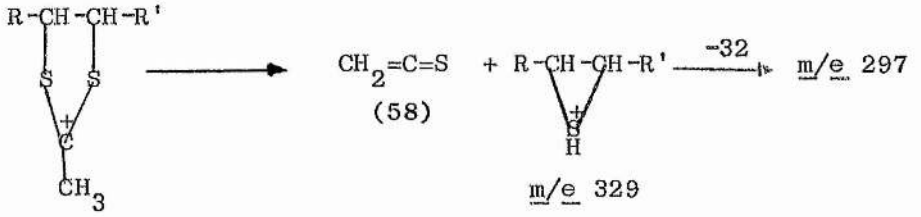
After acetylation this fraction furnished a product which contained a strong absorption band at 1685 ($SCOCH_3$) cm^{-1} in its IR spectrum and a characteristic six-proton signal at 7.72τ ($SCOCH_3$) in its NMR spectrum.

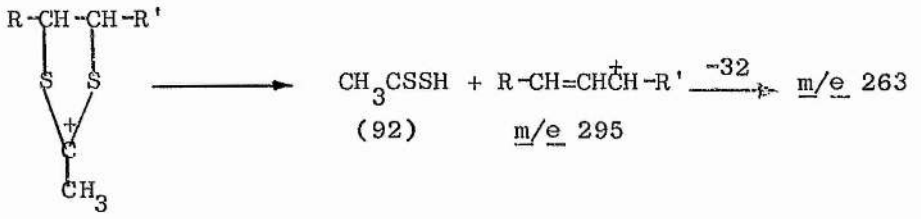
Its identity was confirmed by making its isopropylidene derivative which showed a signal at 6.58-6.65 (m, 2H, $(CH_3)_2C(SCH)_2$)and a six-proton signal at 8.27(s,6H,$(CH_3)_2C(SCH)_2$) in its NMR spectrum. On the basis of the previous study of the cis and trans O-analogues we consider the 9,10-dimercaptostearate to be the threo isomer (for detailed discussion see section 3.1a).

The mass spectrum of the isopropylidene derivative had a molecular ion peak at m/e 402 (16) and a characteristic fragment at 387 (M-15, 38) along with other fragments at 369 (?, 5), 329 (387-58, 21), 328 (329-1, 7), 297 (387-90, 15), 296 (328-32, 3), 295 (387-92, 10), 294 (?, 20), 263 (387-124, 7), 257 (a-32, 1), and 245 (b, 4).

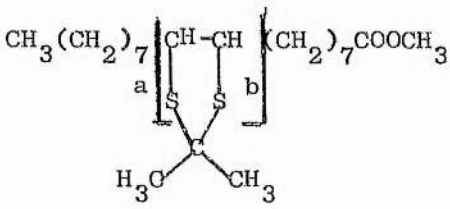
The spectrum indicates that the great majority of the fragments come from the ion 387 (M-15) which results from the

loss of a methyl group from the dioxalane ring. The ions of m/e 297 and 263 result from the loss of thioketene [CH_2=C=S(58)] and thiolacetic acid [$CH_3CS(SH)$ (92)] respectively accompanied in each case by further loss of methanol (32).





The low intensity fragments at m/e 257 (a-32) and 245 (b, 4) resulting from α-cleavage are of considerable structural significance



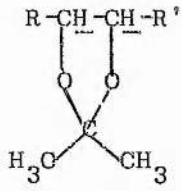
since they indicate the position of the ring.

Band B (20%) with no peak on GLC proved to be a dimer of methyl 9,10-dimercaptostearate. Lithium aluminium hydride reduction of authentic methyl 9,10-dimercaptostearate and of fraction B afforded products which after trifluoroacetylation showed identical behaviour on GLC (ECL 16.5). Both contained infrared absorption bands at 1770 ($OCOCF_3$) and 1700 ($SCOCF_3$) cm^{-1}.

3.1c Methyl erythro-9,10-dimercaptostearate from methyl threo-9,10-dihydroxystearate

The reaction of methyl threo-9,10-dimesyloxystearate with sodium hydrogen sulphide in dimethylformamide solution furnished a product which was separated into two fractions: A (50%) and B (20%) by prep TLC.

Fraction A (50%) is believed to be methyl 9,10-dimercaptostearate. It had a late-running peak of ECL ca 32 on GLC. Its infrared spectrum showed an absorption band at 2580 (SH) cm^{-1} whilst its NMR spectrum contained a two-proton signal at 7.16τ (-CH(SH)CH(SH)). After acetylation fraction A showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum contained a six-proton signal at 7.72τ ($SCOCH_3$). The isopropylidene derivative had signals at 6.20-6.44τ (m, 2H, $(CH_3)_2C(SCH)_2$) and at 8.28τ (m, 6H, $(CH_3)_2C(SCH)_2$-). For the purpose of comparison the methine proton signals of the threo and erythro O and S compounds are set out below. There is a marked difference in the chemical shifts of the methine protons of the threo (6.54-6.66τ) and erythro (6.06-6.20τ) O compounds.

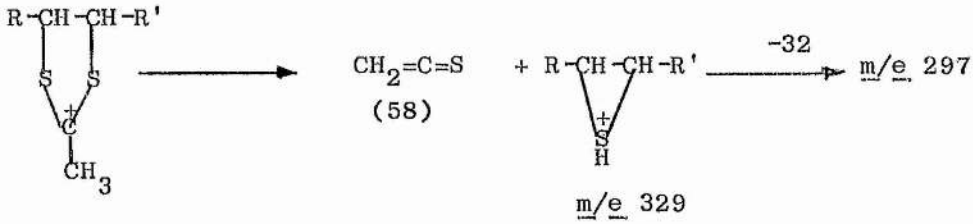


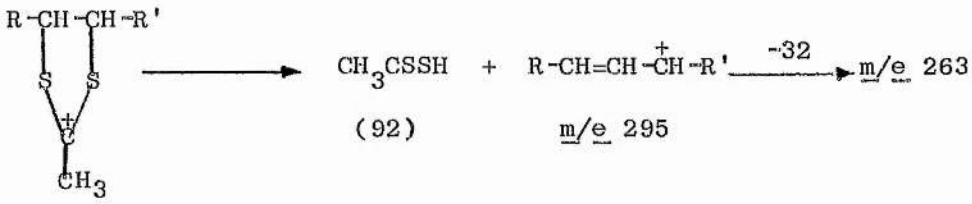
If it is assumed that in the S compounds the higher signal (6.58-6.64τ and 6.20-6.44τ) is in association with the threo isomer then it follows that the erythro diol furnishes the threo dimercaptan and vice versa.

The mass spectrum of the isopropylidene derivative had a molecular ion peak at 402 (20), a characteristic fragment at m/e 387 (M-15, 40), and other peaks at 369 (?, 5), 329 (387-58,

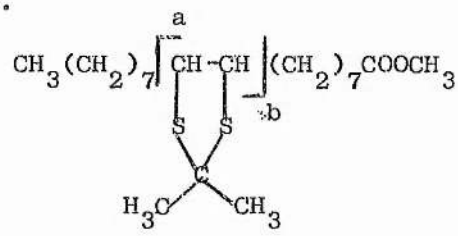
2), 328 (329-1, 8), 297 (387-90, 16), 296 (328-32, 3), 295 (387-92, 10), 294 (?, 20), 263 (387-124, 7), 257 (a-32, 2), and 245 (b, 6).

Like its oxygen analogue the peak at m/e 387 (M-15) resulted from the loss of methyl group from the dioxalane ring. The peaks at m/e 297 and 263 result from the loss of thioketene (58) and thioacetic acid (92) respectively accompanied in each case by further loss of methanol (32).





The low intensity fragments at m/e 257 (a-32) and 245 resulting from α-cleavage are of considerable structural significance.



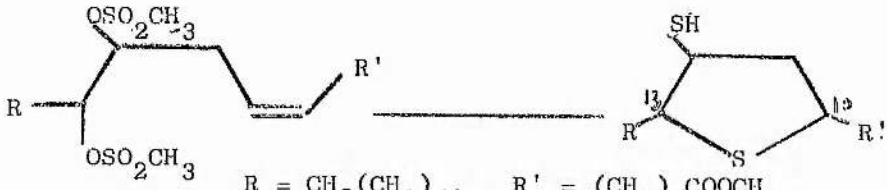
since they indicate the position of the ring.

Fraction B (20%) with no peak on GLC is probably a dimer of methyl erythro-9,10-dimercaptostearate.

3.1d Methyl 10,13-epithio-12-mercaptostearate

Methyl 12,13-dimesyloxyoctadec-cis-9-enoate and sodium hydrogen sulphide in dimethylformamide solution were left at

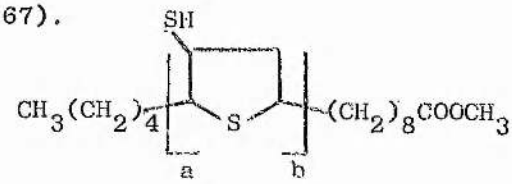
room temperature for 24 hours. The product showed two major bands, A (30%) and B (20%), along with several diffuse polar bands. Only the major bands were isolated for further examination.



R = $CH_3(CH_2)_4$, R' = $(CH_2)_7COOCH_3$

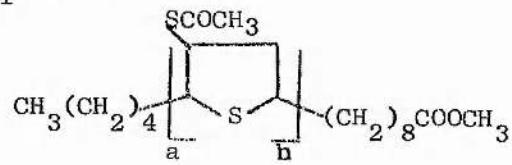
Band A (30%) with a late-running peak of ECL~30 on GLC was believed to be methyl 10,13-epithio-12-mercaptostearate. After acetylation and trifluoroacetylation band A showed strong infrared absorption at 1685 ($SCOCH_3$) and 1700 ($OCOCF_3$) cm^{-1}. The NMR spectrum of fraction A gave characteristic signals at 6.40-6.80 (m, 2H, -CH(S)CH(SH)CH_2CH-) and 6.80-7.20τ (m, 1H, -CH(S)CH(SH)CH_2CH-). In addition its acetyl derivative had a three-proton signal at 7.72τ ($SCOCH_3$).

The mass spectrum of methyl 10,13-epithio-12-mercapto-stearate contained major peaks at: 329 (M-31, 18), 328 (M-32, 20), 327 (M-33, 49), 326 (M-34, 44), 296 (M-64, 22), 294 (M-66, 11), 289 (a, 2), 258 (a-31, 10), 257 (a-32, 44), 225 (a-64, 3), 223 (a-66, 5), 189 (b, 44), 156 (b-33, 7), 155 (b-34, 33) and members of the series $(CH_2)_nCOOCH_3$ (59-157), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).



The spectrum shows that the molecular ion and fragment a can lose 31 (OCH_3), 32 (CH_3OH), 33 (SH) and 34 (H_2S) mass units and combinations of these. Fragment b can lose 33 (SH) and 34 (H_2S) mass units. Fragment a and b clearly indicate the position of the ring.

The mass spectrum of methyl 12-acetylmercapto-10,13-epithiostearate contained major peaks at: 328 (M-74, 18), 327 (M-75, 57), 326 (M-76, 91), 295 (M-107, 15), 293 (?, 13), 203 (?, 10), 270 (?, 39), 257 (a-74, 22), 157 (b-74, 39), 156 (b-75, 13), 155 (b-76, 55) and members of the series $(CH_2)_nCOOCH_3$ (59-157), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).

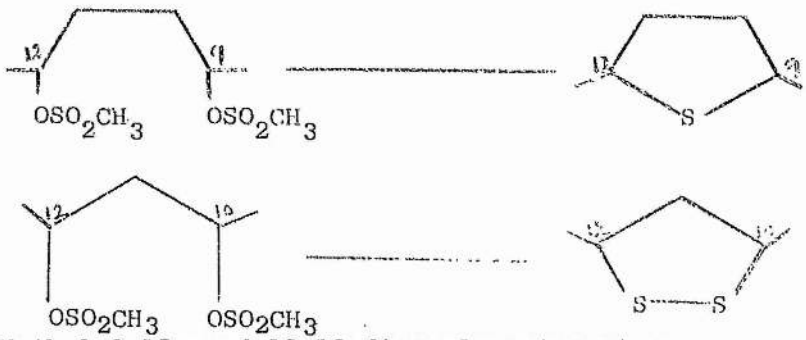


The molecular ion and some of the fragment ions lose further units including CH_3CO (43), CH_3O (31), CH_3OH (32), $SCOCH_2$ (74), $SCOCH_3$ (75) and $HSCOCH_3$ (76). Fragments a and b result from α-cleavage and show the position of the ring.

Band B (20%) showed no peak on GLC and was not examined further. It was possibly the dimer.

3.2 Preparation of methyl 9,12-epithio- and 10,12-epidithiostearates

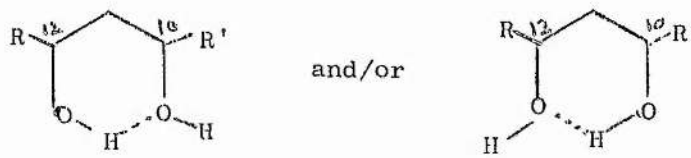
This section concerns the preparation of methyl 9,12- and 10,12-dihydroxystearates from methyl ricinoleate by oxymercuration-demercuration and the reaction of their dimesyloxy esters with sodium hydrogen sulphide. The 9,12-dimesyloxy ester gives methyl 9,12-epithiostearate but the 10,12-isomer yields methyl 10,12-epidithiostearate. The dimercapto esters were obtained by an alternative procedure and each of these can be converted to an epidithiostearate.



3.2a Methyl 9,12- and 10,12-dimesyloxystearates

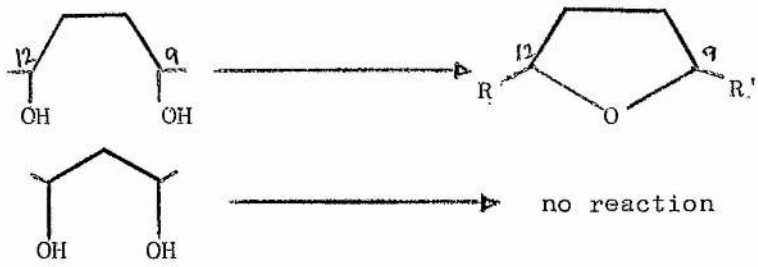
Hydroxymercuration-demercuration of methyl ricinoleate in aqueous tetrahydrofuran (1:1 v/v) gave methyl 9,12- and 10,12-dihydroxystearates (62%), starting material (21%) and methyl 9,12-epoxystearate (17%) [160]. The epoxystearate (ECL 20.9) was identified by chromatographic comparison with authentic material.

The methyl dihydroxystearates were examined on GLC as bis-TMS ethers (ECL 23.2, 12% and 23.4, 88%). TLC (PE70) of the dihydroxy esters showed two spots, the smaller of which was the less polar. This suggests that the minor component was the 10,12-dihydroxy ester which would undergo intramolecular hydrogen bonding as shown:



Similar hydrogen bonding in the 9,12-dihydroxy ester would involve a 7-membered ring.

This tentative identification of the 10,12-dihydroxy ester as the minor component was confirmed by dehydration of the mixture with methanolic sulphuric acid. TLC of the dehydrated product showed the minor spot to be unchanged, whereas the larger spot was replaced by the less polar 9,12-epoxide, formed by the cyclo-dehydration of the 9,12-dihydroxy ester:



Prep TLC of this reaction product gave methyl 10,12-dihydroxy-stearate (12%, ECL 23.2 as bis-TMS ether) and methyl 9,12-epoxystearate (88%, ECL 20.9).

The 9,12- and 10,12-dihydroxystearates were separated, each was,treated with methanesulphonyl chloride. The product in each case was a mixture of the desired dimesyloxy ester (ca 60%) with a six-proton NMR signal at 7.08τ ($-CHOSO_2CH_3$) and a less polar product (ca 40%) with its NMR signal at 7.02τ equivalent only to three protons. This may be a chloromesyloxy ester though this was not proved. Similar results were obtained with butane-1,3-diol and with pentane-2,5-diol.

The less polar bands may be a chloro mesyloxy ester although the chloride was not detected after sodium fusion.

In each reaction of methyl 9,12- and 10,12-dimesyloxy esters therefore, TLC purified material was used and its identity was confirmed by the presence of the six-proton NMR signal at 7.08τ.

3.2b Methyl 9,12-epithiostearate

Reaction of methyl 9,12-dimesyloxystearate with sodium hydrogen sulphide afforded a single component (80%) which was shown to be methyl 9,12-epithiostearate (ECL 24.2) on the basis of the following evidence:

(i) A sample of this component purified by double

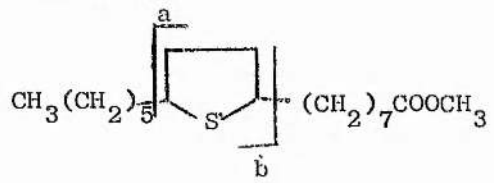
development on prep TLC was analysed: Found: C, 69.58; H, 11.53, calc for $C_{19}H_{36}O_2S$: C, 69.56: H, 10.98%.

(ii) It had the same TLC and GLC behaviour as authentic methyl 9,12-epithiostearate.

(iii) The NMR spectrum showed signals at 6.45-6.95τ (m, 2H, $-CH(S)CH_2CH_2CH-$) along with other usual signals.

(iv) The mass spectrum indicated a molecular weight of 328 which corresponded to a molecular formula $C_{19}H_{36}O_2S$ and which was in agreement with the results obtained from combustion analysis.

The fragments present in the mass spectrum of this component were similar to those in the spectrum of authentic methyl 9,12-epithiostearate. In particular the base peak at m/e 171 is characteristic of methyl 9,12-epithiostearate. Other major peaks were observed at: 328 (M, 38), 297 (M-31, 25), 243 (a, 68), 211 (a-32, 83), 173 (b+2, 4), 172 (b+1, 15), 171 (b, 100), 87 (c, 89), and members of the series $(CH_2)_nCOOCH_3$ (59-143), C_nH_{2n+1} (57-141) and C_nH_{2n-1} (69-139).



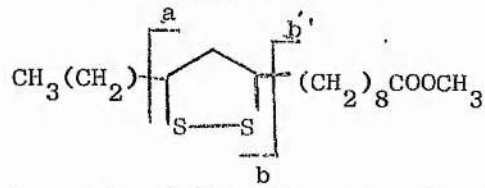
Fragments a and b result from α-cleavage. Fragment a can lose a further 32 mass units presumably from the ester functions. Fragment c is the central unit remaining after two α-cleavage a and b.

3.2c Methyl 10,12-epidithiostearate

Reaction of methyl 10,12-dimesyloxystearate with sodium hydrogen sulphide in the usual way furnished a single component (80%, ECL 24.2) which was shown to be methyl 10,12-epidithiostearate

since it remained unchanged on treatment with acetic anhydride in the presence of anhydrous sodium acetate. The NMR spectrum of the alleged epidithio ester contained a broad signal at 6.44-6.68τ which was believed to be of $-(CH(S)CH_2CH(S))$ whilst its mass spectrum confirmed the molecular weight of 360. Details of other mass spectral fragments are discussed below.

Major peaks at: 362 (M+2, 10), 361 (M+1, 18), 360 (M, 70), 329 (M-31, 10), 328 M-32, 13), 327 (M-33, 9), 297 (M-63, 9), 263 (?, 16), 243 (a-32, 9), 211 (243-32, 8), 199 (?, 15), 189 (b, 3), 185 (?, 12), 171 (b', 10) and members of the series $(CH_2)_nCOOCH_3$ (59-157), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).



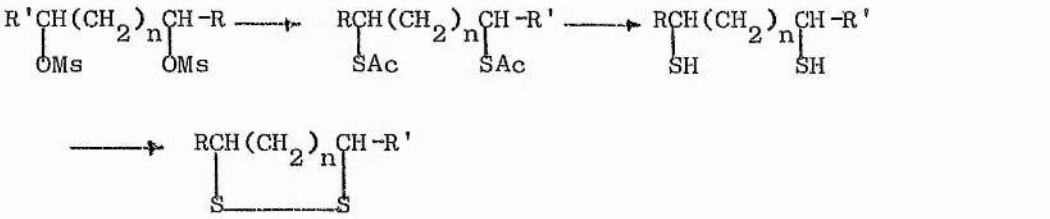
The fragmentation pattern shows that the molecular ion peak loses 31 (OCH_3), 32 (CH_3OH) and 63 (32(s) + 31) mass units. Fragments a and b' can lose a further 32 mass units presumably from the ester function. Further loss of one sulphur atom from the fragment a can result in the peak at m/e 211. Fragment a and b, due to α-cleavage, indicates the position of the ring.

3.3 Preparation of methyl 9,12- and 10,12-dimercaptostearates

The reaction of methyl 9,12- and 10,12-dimesyloxystearates with potassium thiolacetate in dimethylformamide solution at 100° for 3 hours afforded methyl 9,12- and 10,12-diacetylmercapto-stearates in 70% and 55% yields.

Normal deacetylation by methanolic sodium methoxide furnishes epidithio esters, but deacetylation by methanolic hydrochloric

acid in the presence of zinc amalgam and the use of degassed water[26] gave the desired dimercapto esters along with some partially deacetylated products. The dimercapto esters were converted to epidithio esters by oxidation with iodine in ethanol.

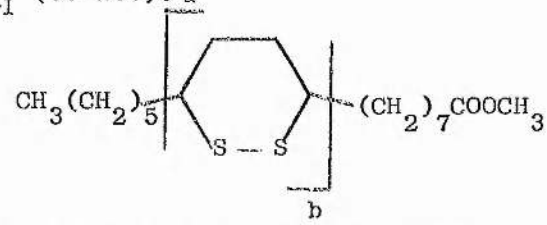


3.3a Methyl 9,12-dimercaptostearate (and methyl 9,12-epidithiostearate)

Reaction of methyl 9,12-dimesyloxystearate with potassium thiolacetate in the usual way gave methyl 9,12-diacetylmercaptostearate (70%). This showed a strong absorption band at 1685 ($SCOCH_3$) cm^{-1} in its IR spectrum and its NMR spectrum gave characteristic signals at 6.44-6.78 [m, 2H, $-CH(SCOCH_3)CH_2CH_2CH(SCOCH_3)-$] and 7.72τ [s, 6H, $-CH(SCOCH_3)CH_2CH_2CH(SCOCH_3)-$].

Deacetylation was accomplished by refluxing with a methanolic solution of sodium methoxide. The product which showed a single spot on TLC and a late-running peak on GLC (ECL ca 30) was believed to be methyl 9,12-epidithiostearate since it did not react with acetic anhydride in the presence of anhydrous sodium acetate and remained unchanged (identity on TLC and GLC) on treatment with iodine in ethanol. Its NMR spectrum showed signals at 7.16-7.28τ (m, 2H, $-CH(S)CH_2CH_2(S)-$) whilst its mass spectrum contained a molecular ion peak at m/e 360 along with other fragments which are detailed below.

Major peaks at: 362 (M+2, 8), 361 (M+1, 7), 360 (M, 31), 329 (M-31, 17), 328 (M-32, 17), 327 (M-33, 31), 297 (M-63, 13), 263 (?, 13), 243 (a-32, 12), 211 (243-32, 12), 171 (b-32, 43), and members of the series $(CH_2)_nCOOCH_3$ (69-157), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).



The fragmentation pattern clearly indicates that the molecular ion peak can lose 31 (OCH_3), 32 (CH_3OH) or 33 (HS) mass units. Fragments a can lose 32 mass units presumably from the ester function. Further loss of one sulphur atom (32) from the fragment a can result in the peak at 211. Fragments a and b due to α-cleavage indicate the position of the ring.

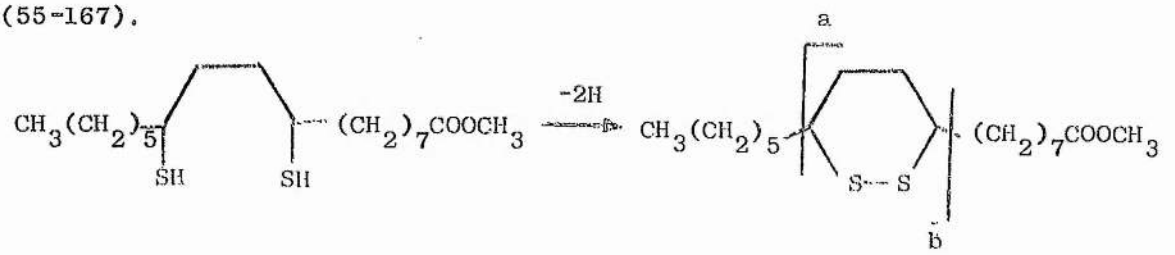
Deacetylation of methyl 9,12-diacetylmercaptostearate by methanolic hydrochloric acid in the presence of zinc amalgam and with the use of degassed water (to reduce the possibility of oxidation of the vicinal dithiol) afforded a product which was separated by prep TLC into two fractions:

Fraction A (52%) showed a late-running peak of ECL (ca 30) on GLC and was believed to be methyl 9,12-dimercaptostearate. Its infrared spectrum showed characteristic absorption band at 2580 (SH) cm^{-1} whilst its NMR spectrum contained a signal at 7.33τ (m, 2H, $-\underline{CH}(SH)CH_2CH_2\underline{CH}(SH)$) with a complete absence of signal at 7.72τ ($SCOC\underline{H}_3$). After reacetylation the fraction showed strong infrared absorption at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum contained a six-proton signal at 7.72τ ($SCOC\underline{H}_3$) .

The dimercapto ester was oxidised by iodine to methyl

9,12-epidithiostearate which remained unchanged on treatment with acetic anhydride and anhydrous sodium acetate and showed a molecular ion peak at m/e 360. It was, however, reduced by lithium aluminium hydride to 9,12-dimercapto-octadecanol. On acetylation this yielded 1-acetoxy-9,12-diacetylmercapto-octadecane whose infrared spectrum showed strong infrared absorption bands at 1685 ($SCOCH_3$) and 1250 (OCH_3) cm^{-1} and its NMR spectrum contained diagnostic signals at 7.72τ (s, 6H, $-CH(SCOCH_3)CH_2CH_2CH(SCOCH_3)-$) and 8.06τ (s, 3H, $-CH_2COCH_3$).

The mass spectrum of the dimercapto ester showed a molecular ion peak at m/e 362 (6) along with other fragments at 360 (M-2, 4), 329 (M-33, or 360-31, 8), 328 (M-34, or 360-32, 15), 297 (328-31, 18), 263 (?, 6), 243 (a-32, 21), 211 (273-32, 27), 171 (b-32, 63), 119 (c, 20), 87 (c-32, 90) and members of the series $(CH_2)_nCOOCH_3$ (59-157), C_nH_{2n+1} (57-169), and C_nH_{2n-1} (55-167).



This spectrum indicates that the molecular ion peak can lose 2 (2H), 31 (OCH_3), 32 (CH_3OH), 33 (SH), 34 (H_2S) and 65 (SH + CH_3OH or H_2S + OCH_3) mass units. The ion of m/e 360 (M-2) can subsequently lose 31 (OCH_3), 32 (CH_3OH) and 32 (S) mass units or one O- and one S-containing fragments. The great majority of the fragments appear to come from the ion 360 (M-2) rather than M.

The fragment a resulting from α-cleavage can subsequently lose a sulphur atom (32) or methanol (32) to result in the ion at m/e 243, or both fragments to give the ion 211. Fragment

b arising from α-cleavage can lose 32 (S) mass units to yield the ion 171. Fragment c, the central unit remaining after the α and β-cleavage, can further lose 32 (S) mass units.

High resolution m/e values for some of the fragment ions confirm their molecular structures:

m/e value	Observed mass	Calculated mass	Formula
360	360.214936	360.215662	$C_{19}H_{36}O_2S_2$
297	297.225915	297.225189	$C_{18}H_{34}OS$
243	243.141131	243.141868	$C_{13}H_{23}O_2S$
211	211.115582	211.115655	$C_{12}H_{19}OS$
171	171.121121	171.120741	$C_{10}H_{19}S$

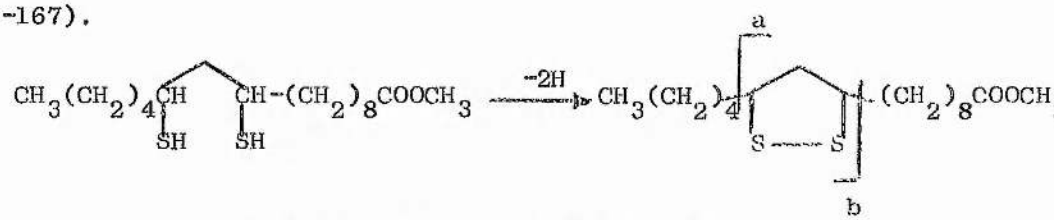
3.3b Methyl 10,12-dimercaptostearate (and methyl 10,12-epidithiostearate

Reaction of methyl 10,12-dimesyloxystearate with potassium thiolacetate in the usual way furnished methyl 10,12-diacetylmercaptostearate (55%). This showed a strong absorption band at 1685 ($SCOCH_3$) cm^{-1} in its IR spectrum and its NMR spectrum gave characteristic signals at 6.42-6.66 [m, 2H, $-C\underline{H}(SCOCH_3)CH_2C\underline{H}(SCOCH_3)$] and 7.78τ [s, 6H, $-CH(SCOC\underline{H}_3)CH_2CH(SCOC\underline{H}_3)$].

Deacetylation by methanolic sodium methoxide furnished methyl 10,12-epidithiostearate which appeared as a single spot on TLC and as a late-running peak on GLC (ECL ca 30). An attempt to acetylate the material failed and a sample of this alleged methyl 10,12-epidithiostearate remained unchanged (identity on TLC and GLC) on treatment with iodine in ethanol. Its NMR

spectrum contained signals at 7.00τ [m, 2H, $-\underline{CH}(S)\underline{CH_2}\underline{CH}(S)-$] whilst its mass spectrum confirmed its identity by showing a molecular ion peak at m/e 360 along with other fragments which have already been discussed fully in section 3.2c.

Deacetylation of methyl 10,12-diacetylmercaptostearate by methanolic hydrochloric chloride in the presence of zinc amalgam and the use of degassed water (to reduce the possibility of oxidation of the resulting thiol) afforded a major product which was believed to be methyl 10,12-dimercaptostearate because it showed a molecular ion peak at m/e 362 in its mass spectrum and formed an isopropylidene derivative with characteristic signals at 6.90-7.26τ [m, 2H, $(CH_3)_2C(\underline{CH}S)_2$] and 8.42τ[s, 6H, $(\underline{CH_3})_2C(CHS)_2$] in its NMR spectrum. Its mass spectrum showed a molecular ion peak at m/e 362 (15), along with other fragments at 360 (M-2, 20), 329 (M-33 or 360-31, 20), 328 (M-34, or 360-32, 13), 297 (328-31, 20), 263 (?, 18), 257 (a-32, 11), 225 (257-32, 12), 157 (b-32, 11), 119 (c, 111), 87 (c-32, 90) and members of the series $(CH_2)_nCOOCH_3$ (59-157), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).



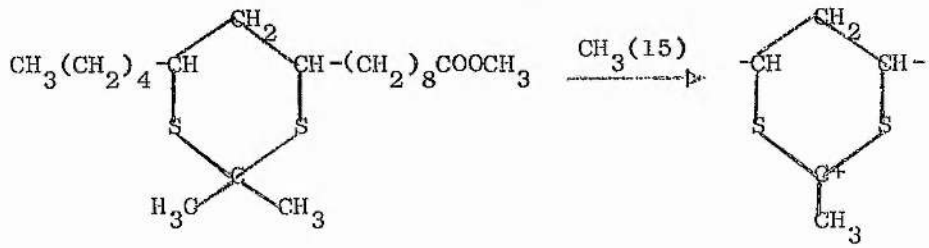
This spectrum indicates that the fragment ions m/e 360 (M-2) can lose 31 (OCH_3), 32 (CH_3OH) and 32 (S) mass units or one O- and one S-containing fragments. The great majority of fragments appear to come from this ion (M-2) rather than M.

The fragment a resulting from α-cleavage subsequently loses 32 mass units (CH_3OH or S) and then a second 32 mass units. Fragment b also due to α-cleavage can lose 32 (S) mass units to yield the ion 157. Fragment c (119), the central unit

remaining after both α-cleavages, can further lose 32 mass units by splitting out a sulphur atom.

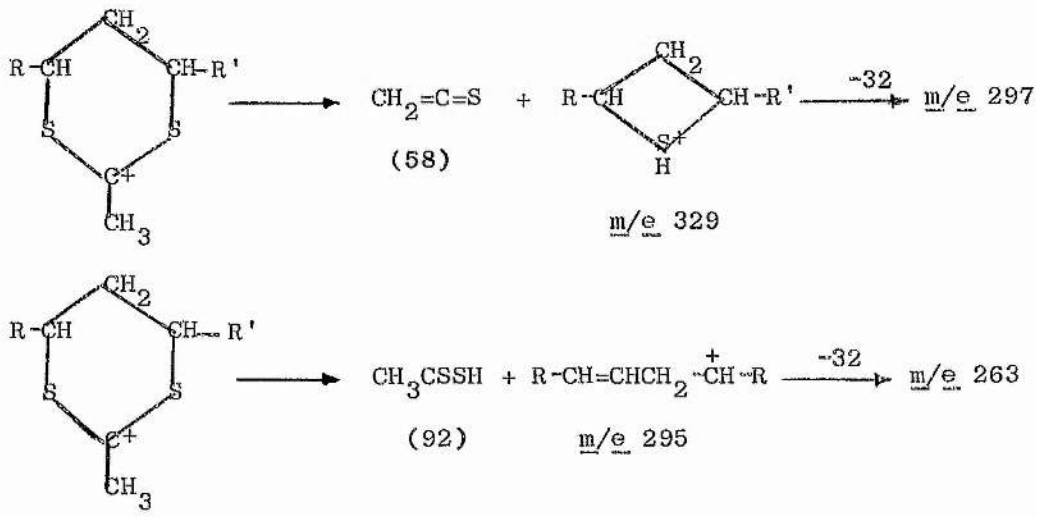
The isopropylidene derivative of methyl 10,12-dimercapto-stearate contained major peaks at 402 (M, 10), 387 (M-15, 3), 329 (387-58, 2), 328 (329-1, 9), 299 (a-32, a), 297 (329-32, 4), 295 (387-92 or 387-58+34, 3), 263 (295-32, 3) and members of the series $(CH_2)_nCOOCH_3$ (59-171), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).

The spectrum shows that the great majority of the fragments appear to come from the ion 387 (M-15) rather than M.



(m/e 387)

The cleavage occurs from the ion 387 by two paths. The peaks at m/e 297 and 263 result from the loss of thioketene [CH_2=C=C(58)] and thioacetic acid [CH_3CSSH(92)] respectively accompanied in each case by further loss of methanol (32).



These fragments are consistent with the expected structure of

the isopropylidene derivative but do not indicate the position of attachment of the S atom. Some evidence for attachment at C(12) may be found in the appearance of a small peak at 299 resulting from α-cleavage followed by loss of 32 (possibly CH_3OH or S) mass units.

3.4 Preparation of αβ-epithio esters

Since the reaction of vicinal dimesyloxy esters with sodium hydrogen sulphide might have resulted in the formation of αβ-epithio esters we found it necessary to know the chromatographic, spectroscopic and chemical behaviour of the αβ-epithio esters.

Several methods are available for the synthesis of aliphatic episulphides from the corresponding epoxides by reaction with sulphur-containing reagents such as thiourea, thiocyanates and substituted thioureas, thioamides and xanthates. For instance, cis-9,10-epithiostearic acid can be prepared by treatment with cis-9,10-epoxystearic acid with thiourea[93] according to the equation

$$R = CH_3(CH_2)_7,\ R' = (CH_2)_7COOH$$

We prepared both methyl cis- and trans-9,10-epithiostearate by reaction of corresponding epoxides with thiourea in the presence of sulphuric acid.

The trans addition of thiocyanogen to olefins has been reported by many workers[80,104,161-163]. We treated methyl oleate with thiocyanogen and obtained methyl threo-9,10-dithiocyanato-

octadecanoate which was converted to cis-9,10-epithiostearate by various reagents.

$R = CH_3(CH_2)_7$, $R' = (CH_2)_7COOCH_3$

We studied the TLC, GLC, NMR and mass spectral behaviour of both cis and trans-epithio esters and found the trans esters to be more stable than the cis in GLC and mass spectrometry. In GLC methyl cis-9,10-epithiostearate decomposed entirely and gave only a peak for methyl octadecenoate whereas the trans isomer was only partially decomposed. The mass spectrum of the trans isomer showed the molecular ion peak whereas the cis-isomer showed no molecular ion peak but only a peak at M-32 (sulphur) even in the 16 ev spectrum.

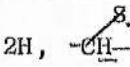
3.4a Methyl cis-9,10-epithiostearate

(i) Reaction of methyl cis-9,10-epoxystearate with thiourea in dioxan solution in the presence of a few drops of conc sulphuric acid gave unreacted epoxide and methyl cis-9,10-epithiostearate (30%). The latter showed a major peak on GLC of ECL 18.5 (with tailing) which presumably arose from methyl octadecenoate formed by decomposition on the column. The NMR spectrum contained a characteristic signal at 7.20τ (m, 2H,  (cis)). Its mass spectrum had major peaks at m/e 296 (M-32, 6), 265 (296-31, 12), and 264 (296-32, 25) and also peaks of the series $(CH_2)_nCOOCH_3$ (59-157), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167). The peak at m/e 296 presumably results from the molecular ion by extrusion of sulphur[164]. It can further lose 31 (OCH_3) or 32 (CH_3OH) mass units.

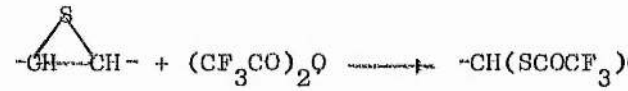
In another experiment cis-9,10-epoxystearic acid, reacted

with thiourea in the presence of conc sulphuric acid in dioxan solution, gave methyl cis-9,10-epithiostearate after esterification.

On treatment with acetyl chloride methyl cis-9,10-epithiostearate gave an acetylmercapto chloro ester[165] whose identity was confirmed from its infrared [a strong absorption band at 1685 cm^{-1} ($SCOCH_3$)] and NMR spectrum [a three-proton signal at 7.70τ ($SCOCH_3$)].

(ii) Methyl threo-9,10-dithiocyanatostearate was prepared from methyl oleate by reaction with thiocyanogen prepared in situ from lead thiocyanate and bromine. This dithiocyanate was converted to methyl cis-9,10-epithiostearate by reacting with several reagents including ethanolic sodium sulphide, methanolic sodium methoxide and lithium borohydride in 50%, 60% and 70% yields respectively. This epithio ester was recognised from its GLC behaviour (ECL 18.5, probably methyl octadecenoate resulting from on-column decomposition) and its NMR spectrum with a characteristic signal at 7.20τ [m, 2H, ].

When the cis-epithio ester was treated with trifluoroacetic anhydride, the product showed strong absorption bands at 1780 ($OCOCF_3$), 1735 ($COOCH_3$) and 1700 cm^{-1} ($SCOCF_3$).



The mass spectrum of the alleged cis-epithiostearate gave major peaks at m/e 296 [M-32 (S)], 264 [M-63 (S + OCH_3)] and 264 [M-64 (S + CH_3OH)].

3.4b Methyl trans-9,10-epithiostearate

Methyl trans-9,10-epoxystearate also reacted with thiourea to afford methyl trans-9,10-epithiostearate (30%). This showed two peaks on GLC of ECL 18.6 (40%, thought to be methyl octadecenoate formed by the partial decomposition in the column) and 24.0 (55%). Its identity was confirmed from its NMR spectrum which contained a diagnostic two-proton signal at 7.54τ [-CH(S)—CH (trans)].

The mass spectrum contained a molecular ion peak at m/e 328 (M, 4), along with other fragments at 297 (M-31, 5), 296 (M-32, 2), 295 (M-33, 3), 294 (M-34, 8), 265 (M-63, 2), 264 (M-64, 5), 263 (M-65, 10), 262 (M-66, 5) and members of the series $(CH_2)_nCOOCH_3$ (59-157), C_nH_{2n+1} (57-169) and C_nH_{2n-1} (55-167).

The spectrum clearly indicates that the molecular ion peak can lose 31 (OCH_3), 32 (CH_3OH or S), 33 (SH), or 34 (H_2S) mass units or combinations of two of these.

Subbaram and Achaya[121] reported that during the desulphurisation of epithio fatty acids there was no change in configuration of the unsaturated acids. We confirmed their claim by the reaction of methyl trans-9,10-epithiostearate with methyl iodide in refluxing acetone to obtain methyl elaidate[166].

$$\underset{\text{trans}}{R\text{-CH(S)—CH-}R'} + 3CH_3I \longrightarrow \underset{\text{trans}}{RCH=CHR'} + I_2 + (CH_3)_3S^+I^-$$

$$R = CH_3(CH_2)_7,\ R' = (CH_2)_7COOCH_3$$

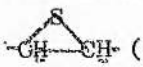
The reaction product was separated into three fractions on prep TLC.

Fraction A (30%, ECL 18.6) was considered to be methyl elaidate since it had a characteristic absorption band at 965 cm^{-1} ($-CH=^{t}CH-$) in its infrared spectrum.

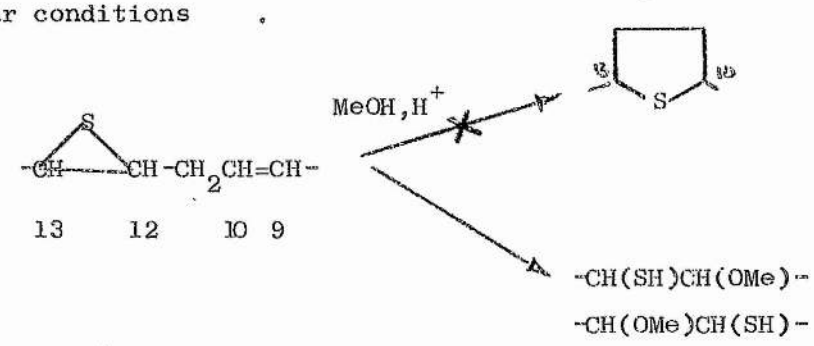
Fraction B (50%) behaved like unreacted methyl trans-9,10-epithio-stearate (identity on TLC and GLC).

Fraction C appeared like a non-lipid material when viewed under the UV lamp and had an obnoxious smell. It was believed to be methyl trimethylsulphonium iodide produced during the reaction.

3.4c Methyl cis-12,13-epithio-octadec-cis-9-enoate

Methyl cis-12,13-epoxyoctadec-cis-9-enoate (methyl vernolate) was treated with thiourea in dioxan solution. The less polar product (35%) showed two peaks on GLC [ECL 19.6 (70%) with tailing which was possibly methyl octadecadienoate formed by the extrusion of sulphur, and 26.2 (30%)], whereas methyl 12,13-epoxyoctadec-cis-9-enoate had ECL of 24.2 only. The NMR spectrum of the alleged epithio alkenoate contained signals at 4.58 (m, 2H, -CH=CH-) and 7.18τ [m, 2H,  (cis)] along with other usual signals.

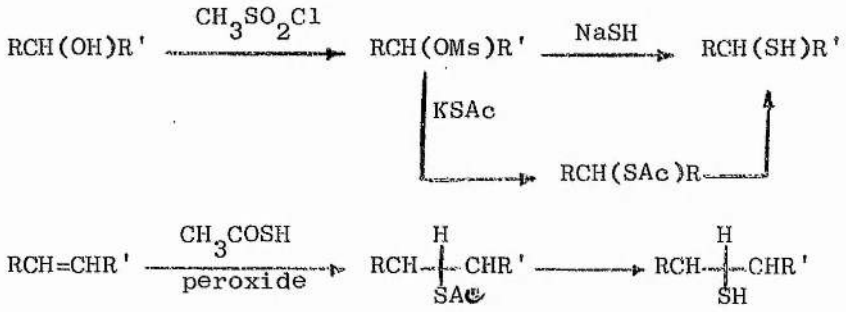
When methyl 12,13-epithio-octadec-cis-9-enoate was refluxed with methanolic sulphuric acid, the major product was a methoxy mercapto alkenoate with a two proton olefinic signal at 4.56τ and a three-proton signal at 6.68τ (OCH_3). There was no evidence of any cyclic sulphide and this observation contrasts with the ready formation of 1,4-epoxides from unsaturated 1,2-epoxides under similar conditions.



4. General comments on methods of preparation and structure determination

4.1a Preparation of monomercapto C_{18} esters

As already indicated we have prepared mercapto ester by three different routes starting from hydroxy esters or from methyl alkenoates.



The reaction between a mesyloxy ester and sodium hydrogen sulphide was effected in dimethylformamide solution at room temperature over 24-30 hours. In alcoholic solution alkoxy esters were also formed. The desired mercapto ester (50-60%) was accompanied by some methyl alkenoate (ca 10%), some dimer (20-30%), and some unreacted mesyloxy ester. We were unable to separate the mercapto ester from the methyl alkenoate except after conversion of the former to its acetyl derivative. This and the ready formation of dimer were obvious disadvantages of this procedure. Using this method, however, we prepared methyl 12-mercaptostearate, methyl 9-mercaptostearate, methyl 12-mercapto-oleate, methyl 12-mercaptoelaidate, methyl 9-mercapto-octadec-cis-12-enoate, 1-mercapto-octadec-4-ene and 1-mercapto-octadec-5-ene. The reaction with methyl 9-mesyloxyoctadec-cis-12-enoate followed a different pathway and is discussed later.

As an alternative route to the mercapto esters we reacted

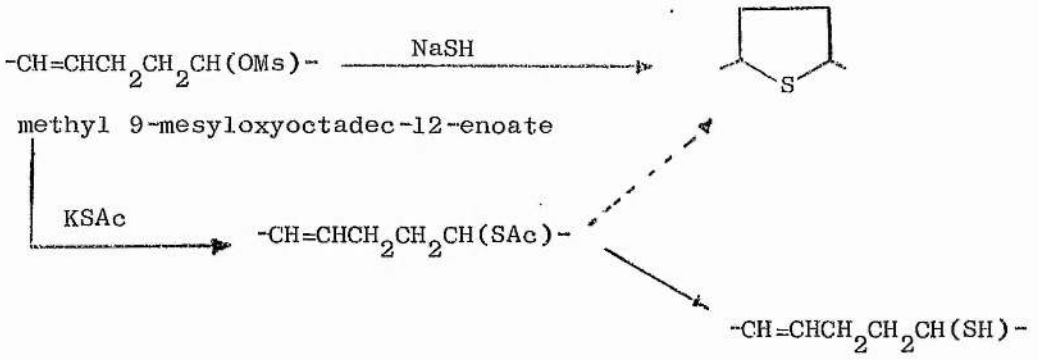
mesyloxy esters with potassium thiolacetate in refluxing acetone (7 hours) or in dimethylformamide at 100°C (2 hours). In this way methyl 12-acetylmercapto-oleate, methyl 12-acetylmercapto-elaidate, methyl 9-acetylmercapto-octadec-cis-12-enoate, 1-acetylmercapto-octadec-4-ene, and 1-acetylmercapto-octadec-5-ene were prepared. Deacetylation with acid or alkali furnished the corresponding mercapto esters in overall yield of 60-70% along with dimer. The formation of this dimer could be avoided by deacetylating in reducing medium (methanolic hydrochloric acid, zinc amalgam and degassed water).

Addition of thiolacetic acid to methyl alkenoates under free-radical conditions afforded methyl acetylmercapto esters (50-60%) as an isomeric mixture along with some polar components which were not identified. Deacetylation of the acetylmercapto esters furnished the corresponding mercapto esters in an overall yield of 45-60%. By this procedure we prepared methyl 9(10)-mercaptostearate, methyl 12-hydroxy-9(10)-mercaptostearate and methyl 9-hydroxy-12(13)-mercaptostearate. Some difficulty was encountered in identifying the individual components of the isomeric mixture since they could not be separated by TLC or GLC and this was an obvious disadvantage of this procedure.

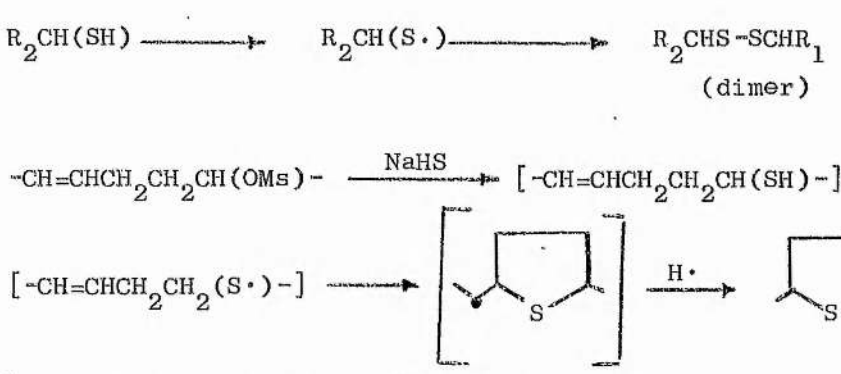
Of these three methods we recommend the reaction of mesyloxy esters with potassium thiolacetate in dimethylformamide solution followed by deacetylation by acid in reducing medium.

When methyl 12-mesyloxyoleate (a β-mesyloxyalkene) reacted with sodium hydrogen sulphide, methyl 12-mercapto-oleate was obtained as expected. Under the same reaction conditions methyl 9-mesyloxyoctadec-cis-12-enoate (a ω-mesyloxyalkene) did not give any mercapto ester but instead gave a cyclic sulphide (methyl 9,12-epithiostearate). The different behaviour of these two

isomeric esters resemble that previously noticed in respect of epoxidation[1] and oxymercuration-demercuration[2] of the corresponding hydroxy octadecenoates when the γ-hydroxyalkene - but not the β-hydroxyalkene - readily furnished an O-heterocycle.



The mercapto ester was obtained via its acetyl derivative so long as the deacetylation was conducted in a reducing medium. We consider the cyclic sulphide to result from a spontaneous oxidation of the intermediate thiol and believe that the dimers, frequently formed during reaction with sodium hydrogen sulphide or during deacetylation of acetylmercapto compound under non-reducing condition , are formed by a similar radical oxidation.



In support of this we were able to isolate some methyl 9-acetylmercapto-octadec-cis-12-enoate from the reaction between the mesyloxyester and NaHS by working up the reaction product quickly and acetylating it immediately. Some acetylmercapto compound was also obtained (25-30%) when the reaction was carried out in an inert atmosphere and in the presence of an antioxidant but we were unable to prevent entirely this intramolecular cyclisation process.

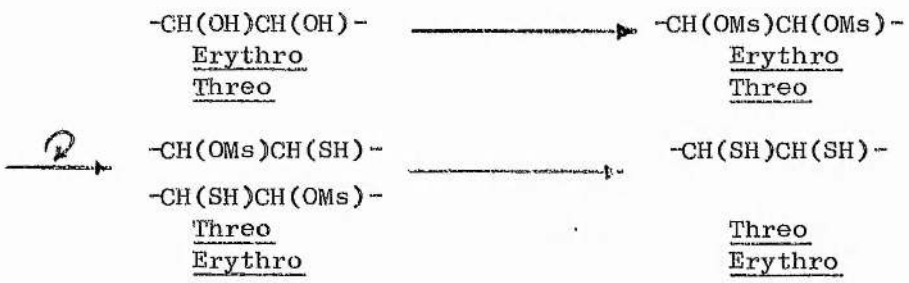
We also found it difficult to keep this methyl mercapto-octadecenoate. Even when stored at 0-5° at inert atmosphere in petrol solution it changed fairly quickly to a mixture of cyclic sulphide and dimer.

4.1b Preparation of dimercapto C_{18} esters

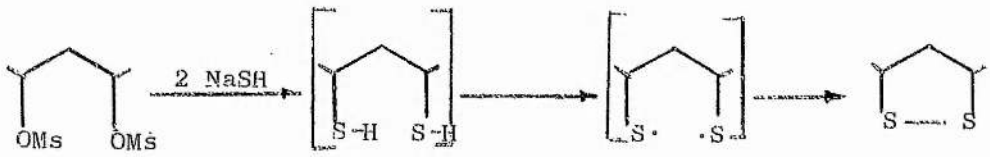
vic-Dihydroxy esters such as methyl 9,10-dihydroxystearate readily form dimesyloxy esters when treated with methanesulphonyl (mesyl) chloride in the usual manner. Our methyl 9,12- and 10,12-dihydroxystearates, on the other hand, gave poorer yields of dimesyloxy ester and had to be purified from less-polar products which may be monochloro-mesyloxy esters.

When methyl erythro and threo- 9,10-dimesyloxystearates, prepared from the corresponding diols, were treated with sodium hydrogen sulphide in the usual way, they were converted to methyl 9,10-dimercaptostearate but there was some evidence that the reaction occurred with overall inversion. Both the vic-dihydroxy esters and the dimercapto esters readily form isopropylidene derivatives. It is known, and we confirmed, that in the NMR spectrum of the cis-dioxalane (from the erythro diol), the methine protons are more deshielded (6.06-6.20τ) than in the trans isomer (6.54-6.66τ). The dimercaptan, obtained from the erythro diol, on the other hand, gives an isopropylidene derivative with methine protons of higher τ value (6.58-6.66τ) than those in the isopropylidene derivative (6.20-6.44τ) of the dimercaptan from the threo diol. This suggests that the dimercaptans from the threo and erythro diols might have been formed through a reaction sequence involving overall inversion. If this is so it can be explained in the terms of neighbouring group participation. The

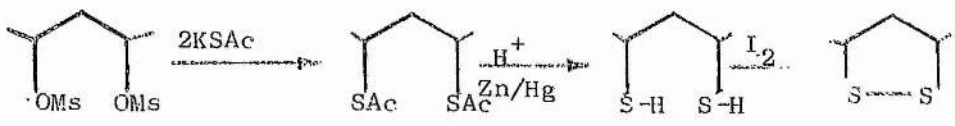
first displacement takes place with inversion as expected of an S_N2 process but the second displacement occurs with retention of configuration through the influence of SH group already present.



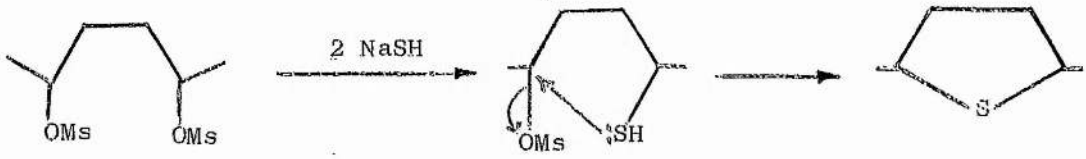
The reaction of methyl 10,12-dimesyloxystearate with sodium hydrogen sulphide furnished methyl 10,12-epidithiostearate as the major product (80%). This is considered to occur by the following scheme involving the intramolecular reaction of a diradical intermediate resulting from the oxidation of the 1,3-dithiol.



Deacetylation of methyl 10,12-diacetylmercaptostearate (produced by the reaction of corresponding dimesylate with potassium thiol-acetate) in a reducing medium furnished the dimercapto ester. Oxidation of these dithiols afforded the epidithiostearate already described.

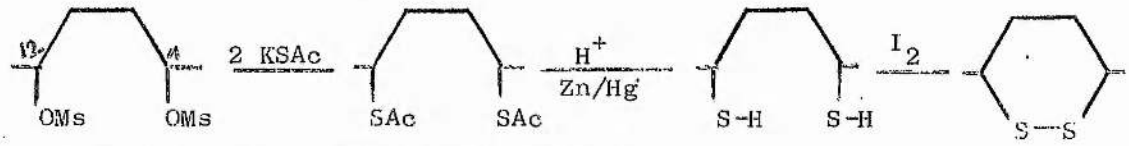


When methyl 9,12-dimesyloxystearate was reacted with sodium hydrogen sulphide, methyl 9,12-epithiostearate was the major product (80%)



This reaction may also be interpreted in terms of neighbouring group participation. After reaction at one centre intramolecular reaction predominates so that the cyclic sulphide is formed in preference to the dithiol.

However, methyl 9,12-dimercaptostearate was obtained by the deacetylation of the corresponding diacetylmercaptostearate in reducing medium. The dimercapto ester on oxidation with iodine furnished the corresponding epidithio ester.



4.2 Methods of structure determination

4.2a Infrared Spectra

The S-H stretching band in the infrared spectra of long-chain monothiols did not prove to be very useful in detecting or identifying these compounds and they were more readily recognised after the preparation of appropriate S-acyl derivatives. In compounds containing two SH groups however - whether vicinal or not - a band of medium intensity was observed at 2560-2580 cm^{-1}.

More useful information was obtained from the infrared spectra of the S-acetyl and S-trifluoroacetyl derivatives. In these compounds C=O stretching bands were observed as follows:

	X=O	X=S
$-XCOCH_3$	1740 cm^{-1}	1685 cm^{-1}
$-XCOCF_3$	1780 cm^{-1}	1700 cm^{-1}
$-COOCH_3$	1730 cm^{-1}	

The bands at 1685 cm^{-1} and 1700 cm^{-1} were used to identify mercapto compounds after conversion to their S-acetyl and S-trifluoroacetyl derivatives.

4.2b Nuclear magnetic resonance spectra

We have examined the NMR spectra of our mono and dimercapto esters and their acetyl derivatives and of the cyclic sulphides we isolated. Significant signals are collected in table 1 and table 2. We found the characteristic singlet at 7.72-7.76τ of the $SCOCH_3$ derivative to be useful in identifying this group and in estimating the purity of those esters which contain three-proton singlets at 6.4τ ($-COOCH_3$) and at 7.7τ ($SCOCH_3$).

The signals associated with the methine protons in the cis and trans three-membered S-heterocycles were in agreement with those reported before[121]. In the ring systems having five or six atoms including one or two sulphur atoms, the signals of the methine protons were of less diagnostic value but showed interesting differences between the five and six-membered ring system. We do not know anything about the configuration of our products containing these rings.

Attention has already been drawn to the difference in the appearance of the methine protons in the cis and trans isomers of the O- and S-isopropylidene derivatives. This evidence suggests - but does not prove - that the erythro diol gave the threo dimercaptan and vice versa.

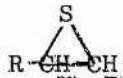
Table 1 NMR of long-chain thiols and their derivatives

X	protons in X^*	$>CH\underline{X}^*$	$-CH_2\underline{X}^*$
SH	[ca 8.7]**	7.15-7.55	-
$SCOCH_3$	6.35-6.45	7.72-7.76	-
$SCOCF_3$	-	6.38	-
$OCOCF_3$	-	-	5.75

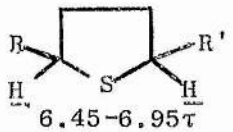
* Quoted values are in τ

** This one-proton signal was not observed at all due to overlap with the broad polymethylene signal around 8.7τ

Table 2 NMR of long-chain cyclic sulphides

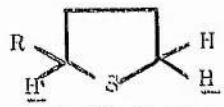
(i) Three membered ring  7.20τ (cis) 7.54τ (trans)

(ii) Five membered ring



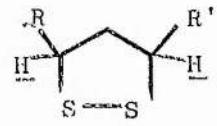
6.45-6.95τ

-



6.66-6.82τ

7.28τ(t)



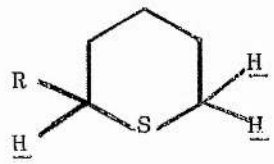
6.44-6.58τ

-

methine

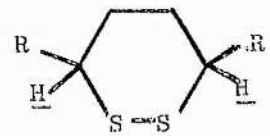
methylene

(iii) Six membered ring



methine 7.38-7.60τ

methylene 8.18τ(t)

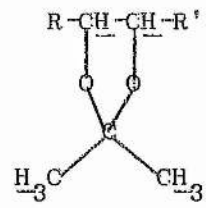


7.16-7.28τ

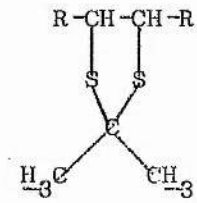
-

(iv)/

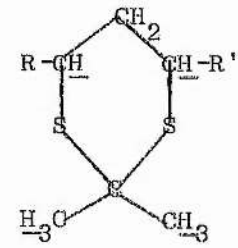
Table 2 (cont)

(iv) Isopropylidene derivatives 

methine protons 6.06-6.20τ (cis), 6.54-6.66τ (trans)
methyl protons [8.7τ]*



methine protons 6.20-6.44τ (cis), 6.58-6.64τ (trans)
methyl protons 8.27τ (S)



methine protons 6.90-7.25τ
methyl protons 8.42τ (S)

* This signal was always hidden by the broad polymethylene signal at 8.7τ but the integral indicated its presence

4.2c Mass Spectra

In considering the mass spectra of our long-chain sulphur-containing compounds we distinguish between those fragments which result from the loss of various functional groups from the C_{18} chain and those in which the C_{18} chain is broken. The former confirm the general structures of our compounds but only the latter indicate the

position of attachment of the functional groups to the main chain.

In addition to loss of 31 (OCH_3), or 32 (CH_3OH) mass units from the ester function our sulphur-containing compounds also lose the following fragments:

Mercapto Compounds HS (33), H_2S (34)

[1,3 and 1,4 dimercapto compounds H_2 (2)]

Acetylmercapto compounds CH_3CO (43), $SCOCH_2^+$ (74), $SCOCH_3$ (75), $HSCOCH_3$ (76)

Isopropylidene derivatives CH_3 (15), $CH_2{=}C{=}S$ (58), CH_3CSSH (92)

[For detailed discussion see sections 3.1a and 3.1b]

Cyclic sulphur compounds (αβ epithio compound) 32 (S)

Five and six membered epidithio compound 32 (S)

In addition, all the spectra are dominated by series of peaks arising from the ions $(CH_2)_nCOOCH_3$, C_nH_{2n+1} and C_nH_{2n-1}. Further details are given in every mass spectral discussion.

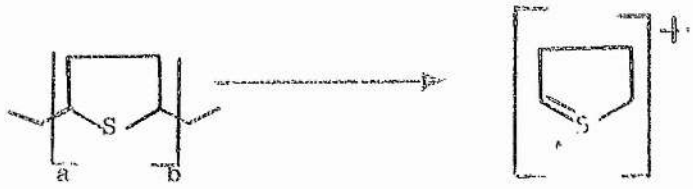
Fragmentation involving cleavage of the C_{18} chain

(i) Mercapto and acetylmercapto esters. Fragments due to α-cleavage indicate the position of -SH and -SAc groups in the chain. Ions containing the SH group may further lose 32 (SH) and 34 (H_2S) mass units and those containing $SCOCH_3$ groups may further lose 42 (CH_2CO), 43 (CH_3CO), 74 ($SCOCH_2^+$), 75 ($SCOCH_3$) and 76 ($HSCOCH_3$) mass units.

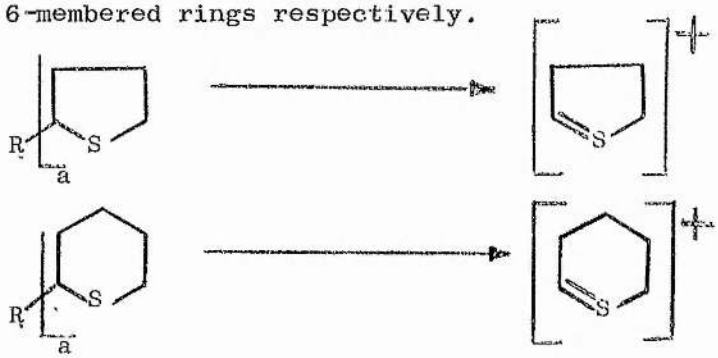
(ii) Three-membered epithio rings. Ions due to α-cleavage were not observed.

(iii) Five or six membered rings. These epithio rings undergo two alternative α-cleavages (a or b) to give fragments which

indicate the position of the heterocyclic ring system.



Cleavage at a and b gives an intense peak of m/e 87. In the monosubstituted compounds peaks at 87 and 101 are indicative of 5 and 6-membered rings respectively.



(iv) Five or six-membered epidithio compounds. α-Cleavage is the principal mode of fragmentation. Ions due to the further loss of one sulphur atom from the epidithio ring was also observed.

4.2d Gas Liquid chromatography

The GLC behaviour of long-chain esters is usually expressed in terms of its equivalent chain length (ECL)[167] and so many of these have been reported that they provide useful information about the structures of unknown esters[168,169]. We report the ECL values of our compounds (DEGS column at 190°) in the following table. Though their ECL did not help in the identification of the products they were useful for their recognition once the products had been identified. Our dimercapto compounds and their derivatives gave late-running peak of ECL around 32.

Table 3 ECL of long-chain sulphur containing compounds (DEGS, 190°)

(i) Monomercapto compounds and their acyl derivatives

	SH	$SCOCH_3$	$SCOCF_3$
Methyl 12-mercaptostearate	23.2	25.4	21.5
Methyl 9-mercaptostearate	23.2	25.5	21.7
Methyl 12-mercapto-oleate	23.7	26.0	-
Methyl 12-mercapto-elaidate	23.9	26.1	-
Methyl 9-mercapto-octadec-*cis*-12-enoate	23.6	26.1	-
1-Mercapto-octadec-4-ene	17.8	21.2	-
1-Mercapto-octadec-5-ene	17.8	21.2	-

(ii) Cyclic sulphur derivatives

Methyl *cis*-9,10-epithiostearate	18.5*	
Methyl *trans*-9,10-epithiostearate	18.6*	and 24.0
Methyl 9,12-epithiostearate	24.2	
Methyl 9,12-epidithiostearate	*ca* 30	
Methyl 10,12-epidithiosteatate	*ca* 30	
2-Tetradecyltetrahydrothiophen	18.6	
2-Tridecyltetrahydrothiopyran	18.1	

* This is considered to be the ECL of the 18:1 ester resulting from decomposition on the column.

EXPERIMENTAL

General procedures

Purification of Solvents

All solvents were reagent grade unless otherwise stated. Diethyl ether, tetrahydrofuran and benzene were dried by standing over calcium chloride. After decantation and distillation, each of these solvents was stored over sodium wire. Dimethylformamide (DMF) was dried[171] by the removal of water as an azeotrope (b.p. 78°) after the addition of benzene. The DMF was subsequently distilled (b.p. 153°) and stored over molecular sieve (type 4Å). Dimethylsulphoxide[172] was distilled from calcium hydride under reduced pressure and stored over molecular sieve 3Å. Dioxan was dried by standing over calcium hydride for a couple of days. After decantation and distillation, it was stored over sodium wire. 1,2-Dimethoxyethane (glyme) was distilled from calcium hydride before use. Pyridine was distilled from potassium hydroxide pellets. Methanol and ethanol were dried by reaction with magnesium and iodine according to Vogel's procedure[173]. Petroleum ether was distilled and the fraction with b.p. 40-60° was used.

Spectroscopic Analysis

Infra-red (IR)

Spectra were recorded on a Perkin-Elmer 257 grating spectrometer. Samples were normally run as 1% solutions in carbon disulphide or carbon tetrachloride using sodium chloride cells of 1 mm path length. Some samples were run as thin films between sodium chloride plates.

Ultra-violet spectra (UV)

Ultra-violet spectra were recorded in methanol or ethanol solution on a Unicam SP800 spectrometer.

Nuclear magnetic resonance (NMR)

Spectra were recorded on 10 or 15% solutions in carbon tetrachloride using a Perkin-Elmer R10 Spectrometer (60 MHz) or a Varian HA100 (100 MHz). When sufficient material was not available, the sample (2-15 mg) was run in a microcell in carbon tetrachloride solution at 100 MHz. Chemical shifts were measured in ppm downfield from internal tetramethylsilane (τ = 10).

Mass spectra (MS)[174,175]

The spectra were obtained at 16 or 70 ev on an AEI MS902 mass spectrometer.

Chromatographic techniques

Thin layer chromatography (TLC)

Analytical TLC was carried out on glass plates (20 cm x 5 cm) with a layer (0.25 mm wet thickness) of silica gel G or of silica gel containing 10 or 15% silver nitrate (Ag^+ TLC)[176].

Preparative TLC (Prep TLC) was carried out on glass plates (20 cm x 20 cm) with a silica layer of 1 mm wet thickness. Preparative silver nitrate TLC is abbreviated to Ag^+ TLC). Sometimes prep TLC plates were pre-cleaned with distilled ether.

Ether-petroleum mixtures were normally used as developing solvents. The abbreviation PE20 indicates a mixture of 20% ether and 80% petrol by volume. Components separated on analytical plates

were made visible by iodine vapour and/or by spraying with a 10% solution of dodecaphosphomolybdic acid in ethanol followed by heating at approx. 120°C for 10 min[177]. Components on preparative plates fluoresced under UV light after the plates had been sprayed with a 0.2% solution of 2,7-dichlorofluorescein in methanol. Bands were scraped off, slurried with ether or methanol and filtered.

In any TLC separation the bands are listed A-D(say) in order of increasing polarity (decreasing Rf value).

Gas-Liquid Chromatography (GLC)

A Pye 104 model 4 chromatograph with a flame ionisation detector was used throughout. Glass columns(5 ft x 4 in i.d.) were packed with Gas Chrom Z (70-80 mesh) coated with 5, 10 or 20% diethylene glycol succinate polyester (DEGS). Samples were injected directly on to the column using a 10 µl SGE syringe with an 11 cm needle. Carrier gas (oxygen free nitrogen) flow rate was varied from 15 to 60 ml/min. Oven temperature was varied from 150 to 190°. Sometimes 3% Apiezon L grease (ApL) coated onto Chromosorb G AW DMCS(80-100 mesh) was used and the normal operating conditions were 210°C with a flow rate of 60 ml/min. Peak areas were measured by one or both of the following methods:

(i) Peak height x peak width at half height

(ii) Peak height x retention time.

Saturated straight-chain methyl esters were used as external standards or as internal standards by co-injection. Retention times are reported as equivalent chain lengths (ECL)[167]. Apparent inconsistencies in ECL values reported in the text were due to deterioration of the polar liquid phase with use. Whenever possible the GLC behaviour of

a reaction product was compared with that of an authentic sample run consecutively.

General Chemical Procedures

Esterification[178]

Esterification on a small scale (< 2 g) was carried out by refluxing the acid for 15-25 minutes with methanolic boron trifluoride-methanol complex (14%, 2-3 ml per g of acid) in dry methanol (10 ml per g of acid). The reaction mixture was cooled, poured into a saturated sodium chloride solution and extracted twice with ether. The combined ether extracts were washed with 5% aqueous sodium bicarbonate solution (x 2) and water (x 2) and dried over anhydrous sodium sulphate.

Large scale esterifications were performed using a methanolic solution of sulphuric acid (0.25M, 5 ml per g of acid). The reaction was effected at room temperature overnight or by refluxing for an hour.

Methanolysis

Glycerides were converted to methyl esters by shaking overnight at room temperature or by refluxing for 30 minutes with dry methanolic sodium methoxide (0.1M). The reaction mixture was carefully acidified, poured into brine and extracted with ether. (Free acids were removed from glycerides before methanolysis by passing through an alumina column using chloroform as solvent.)

Preparation of trimethylsilyl ethers[179]

Long-chain hydroxy compounds were converted into their

trimethylsilyl ethers (TMS ethers) to facilitate examination by GLC. To a solution of hydroxy ester (2-5 mg) in dry pyridine (1 ml), hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) were added. The mixture was then shaken for 30 seconds and allowed to stand for five minutes. After removal of pyridine under vacuum, ether (0.3 ml) was added and the solution (3-5 µl) was injected directly on to a GLC column.

Trifluoroacetylation of mercapto compounds[180]

Freshly distilled trifluoroacetic anhydride (0.5 ml) was shaken with the mercapto compound (1-5 mg) for 30 seconds and then refluxed at 60-70° for 20-30 minutes. The excess anhydride was distilled off under vacuum to leave a residue suitable for GLC examination.

Acetylation of mercapto compounds[16]

The mercapto compound (50 mg) was refluxed with acetyl chloride (0.5 ml) for 2-3 hours. After addition of water (10 ml) the acetylmercapto compound was extracted with ether (2 x 15 ml) and washed with sodium bicarbonate solution (0.05M, 10 ml) and with water (2 x 20 ml). The product (53 mg) was purified by prep TLC (PE25), if required.

Alternatively, the excess of acetyl chloride may be evaporated off and the residue examined directly.

Hydrogenation of mercapto compounds (Mozingo reaction)[181]

The mercapto compound (20 mg) was refluxed with a suspension of freshly prepared Raney Nickel[182] (250 mg) in aqueous ethanol (1:3, 4 ml) for 4-5 hours. The product (14 mg) was recovered by ether extraction (20 ml) after addition of water.

Hydrogenation

Samples (10 mg) in methanol (5 ml), with 10% palladium/charcoal (10 mg) as catalyst, were shaken in a hydrogen atmosphere for one hour or more at room temperature. In the case of mercapto compound, because of the poisoning effect of sulphur on palladium, an excess of catalyst was used and the complete hydrogenation took as long as 3-5 days. The catalyst was removed by filtration and the material recovered in high yield (> 90%), by evaporation of the solvent.

Purdie methylation[183]

Long-chain hydroxy compounds were converted into methyl ethers to facilitate GLC, TLC, and MS examination.

Methyl iodide (1 ml) and freshly prepared silver oxide[184] (5 mg) were added to the material under investigation (10 mg). The reaction mixture was heated under reflux for 3 hours, cooled and then filtered. Excess methyl iodide was removed under vacuum.

Lithium aluminium hydride reduction

Acids, esters and glycerides were converted to long-chain alcohols by reaction with excess lithium aluminium hydride (LAH) in dry ether. To a stirred suspension of LAH (20 mg) in dry ether (2 ml) was added dropwise a solution of lipid material (100 mg) also in dry ether (2-5 ml). After stirring for 10 minutes at room temperature excess hydride was destroyed by the cautious addition of wet ether and then water. Dilute sulphuric acid (20 ml, 2M) was added and the product extracted with ether (2 x 20 ml) and dried over anhydrous sodium sulphate.

Methanesulphonates (mesylates) of hydroxy esters

Mono or dihydroxy ester (1 g, 3 mmole) was dissolved in dry, redistilled pyridine and cooled in ice during the slow addition of redistilled methanesulphonyl (mesyl) chloride (0.7 g, 6 mmole, for monohydroxy; 1.4 g, 12 mmole, for dihydroxy compounds). The temperature was not allowed to rise above 20°C and after addition the mixture was stirred at room temperature for 4 hours. It was again cooled in ice during the slow addition of ice which, after an initial evolution of heat, caused the temperature to drop to 0°C. Hydrochloric acid (50 ml, 2M) was then added slowly with further cooling. The mono or dimesyl derivatives were obtained by extraction with ether. The NMR spectra of these compounds showed strong singlets at 6.4τ ($COOCH_3$) and 7.0τ (SO_2CH_3). Examination of the relative intensities of these signals provided a useful check on the extent of the reaction.

von Rudloff oxidation [185,186]

Oxidative cleavage was used to determine the position of unsaturated centres in long-chain compounds.

An oxidising solution of potassium periodate (22.4 g , 0.0975 mmole) and potassium permanganate (0.4 g, 0.0025 mmole) in one litre of water was used. The unsaturated material (3-5 mg) in distilled t-butanol* (7 ml) and water (1 ml) was shaken overnight with aqueous potassium carbonate (0.35M, 1 ml) and oxidising solution (2 ml).

* Commercial tertiary butanol (700 ml) was first oxidised with a 6% aqueous potassium permanganate solution (ca 50 ml) by heating it at 60°C and rotating it at the same time for several hours. It was then used after distillation.

Excess oxidising agent was destroyed with sulphur dioxide and the solution made alkaline (potassium hydroxide pellets). After most of the solvent had been carefully removed under vacuum the residue was acidified (2M HCl), saturated with sodium chloride solution and extracted with ether (2 x 20 ml). The resulting acids were esterified (boron trifluoride-methanol) and the products analysed by GLC.

To minimise the loss of volatile short-chain products it is important that all ether extracts be carefully evaporated at atmospheric pressure.

Oxymercuration-Demercuration

Excess mercuric acetate (100 mg, 0.31 mmole) and methyl oleate (50 mg, 0.17 mmole) in water (3 ml) and dimethylformamide (5 ml) or tetrahydrofuran were left in a stoppered flask at room temperature for 2 to 4 days.

Excess sodium borohydride (20 mg) dissolved in water (10 ml) was added dropwise with stirring to the oxymercuration reaction mixture at 0^{o} (ice bath). The reaction mixture was stirred and extracted with ether (2 x 20 ml).

1. Preparation of methyl 12-mercaptostearate

Methyl 12-hydroxyoleate (ricinoleate)

Castor oil (10 g) was neutralised by passage through a short column of alumina (100-200 mesh) using chloroform as eluting solvent. Evaporation of the chloroform yielded neutralised oil (9.5 g) which was refluxed for 30 minutes with dry methanolic sodium methoxide (1M, 50 ml). The reaction mixture was poured into water (100 ml), saturated with sodium chloride solution and extracted with ether (2 x 50 ml) to yield the methyl esters of castor oil (8.6 g).

These esters were chromatographed on a column of silica gel (Sorbsil, M-60, 300 g) eluting with 200 ml portions of PE5, PE10, PE15, PE20, PE30, PE40, PE50 and PE60. Methyl ricinoleate (7.9 g), eluted mainly by PE30 and PE40, was adjudged pure by TLC (PE25) and GLC (ECL 26.2).

Methyl 12-hydroxystearate

Neutral castor esters (5 g) in methanolic solution (50 ml) were hydrogenated using 10% palladium charcoal (300 mg) as catalyst. The product was reduced with sodium borohydride (300 mg) before being purified by column chromatography using silica gel as before. Pure methyl 12-hydroxystearate (4.4 g) was eluted mainly by PE30 and PE40 and adjudged pure by TLC (PE25) and GLC [ECL 25.8, 19.4 (TMS ether), 20.1 (TFA ester)].

Methyl 12-mesyloxystearate[117]

Methyl 12-hydroxystearate (2 g, 0.64 mmole) was converted to methyl 12-mesyloxystearate (2.25 g) in the normal way. The identity of the methyl 12-mesyloxystearate was confirmed by chromatographic and spectroscopic means.

(i) Silica chromatography is not very useful as the hydroxy

and mesyloxy ester have very similar Rf values.

(ii) On GLC, the mesyloxy ester decomposed as expected and showed a major peak of ECL 18.5 (presumably 18:1) and a small unidentified component (5%) of ECL 23.3. There was however no evidence of any unreacted hydroxy ester.

(iii) The infra-red spectrum showed strong absorption bands at 1340 and 1170 cm^{-1} (associated with SO_2 group) and complete absence of absorption at 3500 cm^{-1} (OH).

(iv) The NMR spectrum (CCl_4, 60 MHz) showed two sharp three-proton singlets of almost equal height at 6.40 (S, 3H, $-COOCH_3$) and 7.10 τ (S, 3H, $-CHOSO_2CH_3$) along with other signals at 5.40(m, 1H, $-CHOSO_2CH_3$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.70 [br.s, 28H, $-(CH_2)_n-$] and 9.12τ [t, 3H, $CH_3(CH_2)-$].

Methyl 12-mercaptostearate (reaction with sodium hydrogen sulphide)

Sodium hydrogen sulphide[187] (500 mg, 9 mmole) was dissolved in dimethylformamide (DMF, 5 ml) by swirling. When the solution developed an intense green colour, methyl 12-mesyloxystearate (1 g, 3.4 mmole) was added and the solution kept at room temperature for 24-30 hours during which time the colour faded. Water (20 ml) was added and the reaction mixture was extracted with ether (30 ml) and with a second portion (30 ml) after acidifying (sulphuric acid, 2M, 1 ml) the aqueous layer. The product (870 mg), recovered from the combined ethereal extracts, was readily separated into four bands A, B, C, and D by prep TLC (PE25).

Band A (60-65%) gave two peaks on GLC [ECL 18.5 (12%) and 23.2 (85%)]. Attempts to separate these two components were unsuccessful except after acetylation. The crude thiol (50 mg) was acetylated and the product (50 mg) was successfully separated (prep TLC, PE25) into two subfractions. The smaller subfraction A_1 (10%) had an

ECL of 18.5 and the larger subfraction A_2 (90%) of ECL 25.4 . The latter component was considered to be methyl 12-acetylmercaptostearate on the basis of the following evidence.

(i) A sample of subfraction A_2, submitted to Mozingo hydrogenation, gave a product which behaved on TLC (PE25) and GLC like an authentic sample of methyl stearate.

(ii) The infrared spectrum (liquid film) of the acetylated product showed strong absorption band at 1685 cm^{-1} ($SCOCH_3$) not present in the infrared spectrum of subfraction A_2. The NMR spectrum (CCl_4, 60 MHz) showed signals at 6.40 (s, 3H, $-COOCH_3$), 6.40-6.44 (m, 1H, $-CHSCOCH_3$), 7.72 (s, 3H, $-CHSCOCH_3$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.75 (br.s, 28H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

(iii) The acetylmercapto ester was hydrolysed with aqueous methanolic (1:1) potassium hydroxide (1.8M) to give 12-mercaptostearic acid. After esterification (boron trifluoride/methanol), the methyl ester gave a single spot on TLC (PE25) and a single peak on GLC [ECL 23.2, 21.5 (TFA ester)].

(iv) The TFA derivative of methyl 12-mercaptostearate contained a diagnostic absorption band at 1700 cm^{-1} ($SCOCF_3$) in its IR spectrum.

(v) Methyl 12-mercaptostearate showed a molecular ion peak at m/e 328 along with other peaks at 297 (M-31), 296 (M-32), 295 (M-33). Full details have already been given in the Discussion.

Band B (20-30%) gave no peak on GLC. On the basis of TLC, it seemed to be unchanged after treatment with sodium borohydride, but gave methylstearate (ECL 18.0) when submitted to hydrogenolysis (Mozingo). This fraction was proved to be the dimer of methyl

12-mercaptostearate by carrying out the following experiments.

(i) Fraction B, reduced with lithium aluminium hydride, gave a product which showed strong infrared absorption band at 3500 cm^{-1} (OH). The trifluoroacetylated product had an ECL of 21.0 and its infrared spectrum (liquid film) contained absorption bands at 1780 ($OCOCF_3$) and 1700 cm^{-1} ($SCOCF_3$). A parallel result was obtained when these experiments were carried out with methyl 12-mercapto-stearate.

(ii) When methyl 12-mercaptostearate was oxidised[27] by keeping it over an ethanolic solution of iodine (0.05M) at room temperature overnight the product showed the same Rf value as fraction B. After reduction of this oxidation product with lithium aluminium hydride followed by the purification on prep TLC (PE25) and then trifluoro-acetylation, the product had an ECL of 21.0. Its infrared spectrum (liquid film) contained absorption bands at 1780 ($OCOCF_3$) and 1700 cm^{-1} ($SCOCF_3$). Its NMR spectrum (CCl_4, 100 MHz) showed signals at 5.75 (t, 2H, $-C\underline{H}_2OCOCF_3$), 6.38 (m, 1H, $-C\underline{H}SCOCF_3$), 8.72 (br.s, 28H, $-(C\underline{H}_2)_n-$) and 9.12τ (t, 3H, $C\underline{H}_3CH_2-$).

Fraction C (6%) was probably unreacted mesylate. On GLC it gave a major peak of ECL 18.5 (90%) and a minor peak of ECL 23.3 (8%).

Fraction D (10%) This most polar fraction gave no peak on GLC even after methylation (boron trifluoride/methanol) and was not examined further.

Methyl 12-mercaptostearate (reaction with potassium thiolacetate)

Methyl 12-mesyloxystearate (130 mg, 0.33 mmole) was refluxed with potassium thiolacetate[25] (250 mg, 2.1 mmole) in dry acetone

(10 ml) for 7.5 hours. After the evaporation of solvent, the crude ester was diluted with water and then extracted with ether [2 x 20 ml, a second extract was obtained after acidifying the aqueous organic layer with hydrochloric acid (2M)]. The product (125 mg) showed a single spot on TLC (PE25). Purification by prep TLC (PE25) afforded a product (82 mg, 68%) which was considered to be methyl acetylmercaptostearate on the basis of its GLC, IR and NMR. GLC showed a single peak of ECL 25.4. The infrared spectrum (liquid film) contained the diagnostic strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and the NMR spectrum (CCl_4, 100 MHz) showed the following signals: 6.40 (s, 3H, $-COOCH_3$), 6.38-6.42 (m, 1H, $-CHSCOCH_3$), 7.72 (s, 3H, $-CHSCOCH_3$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.74 (br.s, 28H, $(CH_2)_n$) and 9.12τ (t, 3H, CH_3CH_2-).

Methyl 12-acetylmercaptostearate (56 mg) was refluxed with methanolic sulphuric acid (1M, 2ml) for 2.5 hours. The reaction mixture was cooled and diluted with water. It was then extracted with ether (2 x 20 ml) and dried. The crude thiol ester (55 mg) showed a major spot on TLC (PE25) along with, a minor spot (possibly the dimer formed during acid hydrolysis) at low Rf. After purification by prep TLC (PE25) the product was examined by GLC, IR, and NMR. GLC showed a single peak of ECL 23.2. The NMR spectrum (CCl_4, 60 MHz) contained signals at 6.40 (s, 3H, $COOCH_3$), 7.20-7.55 (m, 1H, -CHSH), 7.78 (t, 2H, CH_2COOCH_3), *8.75 (br.s, ca 29H, $(CH_2)_n$) and 9.12τ (t, 3H, CH_3CH_2-). The TFA derivative (ECL 21.5) showed a strong absorption band at 1700 cm^{-1} ($SCOCF_3$).

* The single proton signals of -CHSH were always hidden by the polymethylene broad singlet at 8.75τ[188].

Other attempts to prepare 12-mercaptostearic acid

The following experiments, carried out in an attempt to obtain pure mercapto acid free of the contaminating 18:1 acid, were less successful than the acetylation procedure described in the previous section.

(i) Methyl 12-mesyloxystearate (1.27 g, 3.24 mmole) was reacted with sodium hydrogen sulphide (0.75 g, 13.4 mmole) in dimethylformamide (12 ml) at room temperature for 24-30 hours. The product (1.10 g) was heated at 100°C with sodium hydroxide (0.5 g) in methanol (10 ml). The organic acid, recovered after acidification (2M hydrochloric acid, 5 ml), was crystallised from petroleum ether (40-60). A pale yellow solid (500 mg, m.p. 58-59°) was obtained but this showed no significant peaks on GLC after remethylation (boron trifluoride/methanol).

(ii) Methyl 12-mesyloxystearate (583 mg, 1.5 mmole) and thiourea (160 mg, 2.1 mmole) in dimethylformamide were heated at 100°C for 5 hours . The product was hydrolysed by refluxing with sodium hydroxide (300 mg) in water (5 ml). After cooling, the aqueous layer was acidified with sulphuric acid (2M) and then extracted with ether (2 x 20 ml). The recovered acid (480 mg) was crystallised from petroleum ether (40-60). The amorphous colourless solid (m.p. 68-69°C) gave no significant peaks on GLC after remethylation (boron trifluoride/methanol).

2. Preparation of methyl 9-mercaptostearate

Methyl 9-hydroxyoctadec-cis-12-enoate[118]

Strophanthus sarmentosus seed oil (212 g) was refluxed with a solution of potassium hydroxide (62.5 g) in water (300 ml) and methanol (300 ml) for 1 hour. After extraction of unsaponifiable material, the solution was acidified (3M, hydrochloric acid) and then extracted with ether to yield mixed acid (207 g).

The mixed acid (50 g) was dissolved in petroleum ether (40-60, 500 ml) in a separating funnel; three further funnels contained petroleum (40-60, 500 ml). Aqueous methanol (80%, 500 ml) was added to the first funnel and after equilibration, passed through each of the other three funnels in turn. After extraction with five separate portions of 80% methanol, the combined alcoholic extracts were evaporated and the residue, extracted with ether, yielded hydroxy acids concentrate (7.43 g).

Methylation was achieved by refluxing the acids for one hour with methanolic sulphuric acid (0.25M, 25 ml) and the extracted esters (7.30 g) were purified by column chromatography (Sorbsil M-60). Methyl 9-hydroxyoctadec-*cis*-12-enoate (5.5 g), eluted mainly by PE30 and PE40, was adjudged pure on TLC (PE25) and GLC (ECL 26.1).

Methyl 9-mesyloxystearate

The unsaturated ester was hydrogenated as already described to furnish methyl 9-hydroxystearate (ECL 25.8, TMS derivative 19.4, TFA derivative 20.0).

Methyl 9-mesyloxystearate was prepared from the hydroxy ester as already described.

Methyl 9-mercaptostearate

Methyl 9-mesyloxystearate (100 mg, 0.25 mmole) was added dropwise to a solution of sodium hydrogen sulphide (60 mg, 1.07 mmole) in dimethylformamide (3 ml). The mixture was swirled and kept at room temperature for 27 hours. The addition of water, followed by ethereal extraction [2 x 20 ml, the second portion after acidification with sulphuric acid (2M, 2 ml)] yielded a product (80 mg) which gave four bands (A-D) in prep TLC (PE25).

Band A (60-64%) gave two peaks on GLC [ECL 18.6 (12%) and 23.2 (85%)] and its two components were separated by prep TLC (PE25) after acetylation. The larger subfraction (ECL 25.5) was considered to be 9-acetylmercaptostearate on the basis of the following evidence:

(i) The infrared spectrum contained a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) not present in the infrared spectrum of band A.

(ii) The NMR spectrum (60 MHz, CCl_4) showed signals at 6.40 (s, 3H, $-COOCH_3$), 6.40-6.44 (m, 1H, $-CHSCOCH_3$), 7.72 (s, 3H, $-CHSCOCH_3$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.75 (br.s, 28H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2).

(iii) The acetylmercaptoester was hydrolysed with aqueous methanolic (1:1) potassium hydroxide (1.8M) to give 9-mercapto-stearic acid. After esterification (boron trifluoride/methanol), the methyl ester gave a single spot on TLC (PE25) and a single peak on GLC [ECL 23.2, TFA derivative 21.7].

(iv) The TFA derivative of methyl 9-mercaptostearate contained a diagnostic absorption band at 1700 cm^{-1} ($SCOCF_3$).

(v) The major peaks of the mass spectral fragments of methyl 9-mercaptostearate and its acetyl derivative have already been given in the discussion.

Band B (25%) gave no peak on GLC and was considered to be the dimer of methyl 9-mercaptostearate. On treatment with sodium borohydride it remained unchanged as was shown on TLC, but Mozingo hydrogenolysis furnished methyl stearate (ECL 18.0).

A sample of authentic methyl 9-mercaptostearate was oxidised by keeping it over an ethanolic solution of iodine (0.05M) overnight. The recovered sample showed identical polarity with band B and the oxidised sample showed no peak on GLC.

Band C (6%) was probably the unreacted mesylate: It had the same polarity as mesylate and on GLC this fraction gave a major peak of ECL 18.6 (90%, possibly 18:1) and a minor peak of ECL 23.4 (7%).

Band D (ca 10%) This most polar fraction gave no peak on GLC even after methylation (boron trifluoride/methanol) and was not examined further.

3. Preparation of methyl 12-mercapto-oleate

Methyl 12-mercapto-oleate (reaction with sodium hydrogen sulphide)

Methyl 12-mesyloxyoleate (500 mg, 1.3 mmole), prepared from methyl ricinoleate in the usual way, was added slowly to a solution of sodium hydrogen sulphide (300 mg, 5.5 mmole) in dimethylformamide (4 ml) and the mixture was kept at room temperature for 24-30 hours. After addition of water (20 ml) and ether extraction (2 x 30 ml), the product (425 mg) was separated by prep TLC (PE25) into four bands (A-D).

Band A (60-65%) gave four peaks on GLC of ECL 18.8, 20.0, 20.3 and 23.7. Prep TLC pure band A (252 mg) was acetylated and the product was successfuly separated by prep TLC (PE25) into two subfractions A_1 (37 mg) and A_2 (231 mg). A_1 had ECL of 18.8, 20.0 and 20.3 and A_2 of 26.0.

Subfraction A_2 (150 mg) was hydrolysed by boiling with aqueous methanolic(1:1) potassium hydroxide (1.8M, 1.5 ml) for 2.5 hours. Acidification (2M, hydrochloric acid, 5 ml) followed by ether extraction gave 12-mercapto-oleic acid (123 mg) which was esterified to give methyl 12-mercapto-oleate (124 mg, ECL 23.7). The structure is based on the following observations.

(i) A deacetylated sample of subfraction A_2 submitted to Mozingo hydrogenolysis to give a product which behaved like an authentic sample of methyl stearate.

(ii) The acetylated product showed an absorption band at 1685 cm^{-1} (C=O stretching of thioester group) not present in the IR spectrum of subfraction A_2. The spectrum also showed no evidence of any absorption due to trans unsaturation.

(iii) The NMR spectrum (CCl_4, 60 MHz) of methyl 12-mercapto-oleate showed the following signals: 4.62 (m, 2H, -CH=CH-), 6.40 (s, 3H, $-COOCH_3$), 7.10-7.40 (m, 1H, -CHSH), 7.60-80 (m, 4H, $-CH_2CH=CHCH_2-$), 7.78 (t, 2H, $-CH_2COOCH_3-$), *8.65 (br.s, ca, 20H, $-(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-). The acetyl mercapto ester contained the same signals as above except for the signals at 6.45-6.80 (m, 1H, $-CHSCOCH_3$) and an additional signal at 7.72τ (s, 3H, $SCOCH_3$).

(iv) After von Rudloff oxidation followed by methylation (boron trifluoride/methanol) the product showed a GLC peak for methyl nonanedioate as the only dibasic acid ester.

Band B (25-30%) gave no peak on GLC. On the basis of TLC it seemed to be unchanged after treatment with sodium borohydride, but gave methyl stearate (ECL 18.0) when submitted to hydrogenolysis (Mozingo). This fraction was proved to be the dimer of methyl 12-mercapto-oleate by carrying out the following experiments.

(i) Fraction B, reduced with lithium aluminium hydride, gave a product which showed strong absorption band at 3500 cm^{-1} (OH). Its TMS-derivative showed two peaks of ECL 20.9 (70%) and 19.9 (ca 20%). The trifluoroacetylated product had an ECL of 20.3 and its infrared spectrum (liquid film) showed absorption bands at 1780 ($OCOCF_3$) and 1700 cm^{-1} ($SCOCF_3$).. The NMR spectrum (CCl_4, 100 MHz) contained signals at 4.62 (m, 2H, $-CH_2CH=CHCH_2-$), 5.70 (t, 2H, $-CH_2OCOCF_3$), 6.35 (m, 1H, $-CHSCOCF_3$), 7.62 (dd, 2H, $-CH(SCOCF_3)CH_2CH=CH-$), 8.00 (m, 2H, $-CH=CHCH_2-$), 8.62 (br.s, ca 21H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-). A similar product was

* The single proton of (-CHSH) was always hidden by polymethylene broad singlet at 8.65τ.

obtained by lithium aluminium hydride reduction of methyl 12-mercapto-oleate.

(ii) Methyl 12-mercapto-oleate was oxidised with iodine in ethanol solution (0.05M) by keeping the mixture at room temperature overnight. The isolated product showed the same Rf value as fraction B. After reduction with lithium aluminium hydride followed by purification on prep TLC (PE25) and then trifluoroacetylation, the product had an ECL of 20.3. The TMS-derivative had ECL of 20.9 (70%) and 19.9 (20%). The infrared spectrum (liquid film) of the trifluoroacetylated product showed absorption bands at 1780 ($COOCH_3$) and 1700 cm^{-1} ($SCOCF_3$). The NMR spectrum (CCl_4, 100 MHz) contained the same signals as those reported in (i).

Band C (4%) was probably unreacted mesylate . On GLC it gave a series of peaks at 19.4, 20.3, 20.8 and 23.3 which are thought to arise from on-column decomposition of the ester.

Band D (ca 10%) gave no peak on GLC even after methylation (boron trifluoride/methanol) and was not examined further.

Methyl 12-mercapto-oleate (reaction with potassium thiolacetate)

Methyl 12-mesyloxyoleate (170 mg, 0.43 mmole) and potassium thiolacetate (228 mg, 2 mmole) were heated at 100°C in dry dimethylformamide for 3 hours. After cooling, water was added and the reaction mixture was extracted with ether [2 x 20 ml, the second portion was extracted after acidifying the aqueous organic layer with hydrochloric acid (2M)]. The product (162 mg) showed a single spot on TLC (PE25). Purification by prep TLC (PE25) afforded a product (112 mg, 70%) which was considered to be methyl

12-acetylmercapto-octadec-cis-9-enoate on the basis of its GLC, IR and NMR. GLC showed a single peak of ECL 26.0, the infrared spectrum (liquid film) contained the diagnostic strong absorption band at 1685 cm^{-1} ($SCOCH_3$). The NMR spectrum (CCl_4, 100 MHz) showed signals at 4.62 (m, 2H, $-CH=CH-$), 6.40 (s, 3H, $-COOCH_3$), 6.45-6.80 (m, 1H, $-CHSCOCH_3-$), 7.60-8.00 (m, 4H, $-CH_2CH=CHCH_2-$), 7.72 (s, 3H, $CHSCOCH_3$), 7.78 (t, 2H, $-CH_2COOCH_3-$), 8.65 (br.s, 21H, $(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-).

Methyl 12-acetylmercapto-octadec-cis-9-enoate (87 mg) was refluxed with methanolic sulphuric acid (1M, 3 ml) for 2.5 hour. The mixture was cooled and diluted with water. It was then extracted with ether (2 x 20 ml) and dried (Na_2SO_4). The crude thiol ester (81 mg) showed a major spot on TLC (PE25) along with a minor spot (possibly the dimer formed during acid hydrolysis) at lower Rf. After purification by prep TLC (PE25) the product was examined by GLC and NMR. GLC showed a single peak of ECL 23.7. The NMR spectrum (CCl_4, 100 MHz) contained signals at 4.62 (m, 2H, $-CH=CH-$), 6.40 (s, 3H, $-COOCH_3$), 7.10-7.40 (m, 1H, $-CHSH-$), 7.60-8.00 (m, 4H, $-CH_2CH=CHCH_2-$), *8.65 (br.s, ca 21H, $(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

* The single proton of $-CHSH$ was always hidden by the polymethylene broad singlet at 8.65τ

4. Preparation of methyl 12-mercapto-octadec-trans-9-enoate

Methyl 12-mercapto-octadec-trans-9-enoate (reaction with sodium hydrogen sulphide)

Methyl 12-mesyloxyoctadec-trans-9-enoate (90 mg, 0.23 mmole) prepared from methyl ricinelaidate[119] in the usual way, was added to a solution of sodium hydrogen sulphide (50 mg, 0.88 mmole) in dimethylformamide (3 ml). The mixture was swirled and kept at room temperature for 24 hr. After the addition of water, the reaction product was extracted with ether [2 x 20 ml, the second extract, after acidifying the aqueous organic layer with a few drops of sulphuric acid (2M)]. The combined ethereal extracts were dried and the product (68 mg) was separated by prep TLC (PE25) into three components of which only the least polar was examined.

This band gave several peaks on GLC ECL (19.6, 20.3, 22.3 and 23.9) of which the last one was the largest. After acetylation the product (72 mg) had a different behaviour on TLC and its main GLC peak was at ECL 26.1. The infrared spectrum (liquid film) contained characteristic absorption bands at 970 ($-CH\overset{t}{=}CH-$), 1685 ($SCOCH_3$) and 1735 cm^{-1} ($-COOCH_3$).

The crude methyl 12-acetylmercapto-octadec-trans-9-enoate (48 mg, ECL 26.1) was refluxed with methanolic sulphuric acid (1M, 5 ml) for 2 hours. The recovered product was examined by TLC (PE25) and GLC. TLC (PE25) showed several spots, but the major one was possibly the dimer of methyl 12-mercapto-elaidate (formed by oxidation during acid hydrolysis). GLC showed several peaks of ECL 19.6, 20.3, 22.3 and 23.9 (40%) and 24.2 (5%) with the complete absence of peak of ECL 26.1. The last peak of ECL 24.2 was possibly methyl 9,12-epithiostearate. Further studies to prove its structure

were not possible due to lack of material and its contamination with other compounds.

Methyl 12-acetylmercapto-octadec-trans-9-enoate (reaction with potassium thiolacetate)

Methyl 12-mesyloxyoctadec-trans-9-enoate (100 mg, 0.25 mmole) and potassium thiolacetate (114 mg, 1 mmole) were heated at 100°C in dry dimethylformamide for 3 hours. The crude product (112 mg) showed a single spot on TLC (PE25). Purification by prep TLC (PE25) afforded a product which was considered to be methyl 12-acetylmercapto-octadec-trans-9-enoate by GLC and IR. GLC had a single peak of ECL 26.1 and its IR spectrum (liquid film) contained absorption bands at 970 cm^{-1} ($-CH=\overset{t}{C}H-$), 1685 ($SCOCH_3$) and 1735 cm^{-1} ($COOCH_3$).

Attempted acid catalysed cyclisation of methyl 12-acetylmercapto-octadec-trans-9-enoate and cis-9-enoate

(i) Methyl 12-mercapto-octadec-cis-9-enoate (45 mg) was refluxed with methanolic sulphuric acid (1M, 5 ml) for 2 hr. The reaction mixture was cooled, diluted with water, then the aqueous organic layer was extracted with ether. The product showed a major spot on TLC (PE25) which was possibly the dimer of methyl 12-mercapto-octadec-cis-9-enoate, formed by oxidation during acid hydrolysis, along with a minor spot of methyl 12-mercapto-octadec-cis-9-enoate. [Similar Rf to that of authentic methyl 12-mercapto-octadec-cis-9-enoate on TLC (PE25)]. GLC had a single peak of ECL 23.7 (identical with that of authentic methyl 12-mercapto-oleate).

(ii) Methyl 12-mercapto-octadec-trans-9-enoate (50 mg) was refluxed with methanolic sulphuric acid (1M, 5 ml) for 2 hours. Working up as above afforded a product which showed two spots on TLC (PE25), the more polar major one was possibly the dimer of

methyl 12-mercapto-octadec-trans-9-enoate, and the minor one, methyl 12-mercapto-octadec-trans-9-enoate. GLC of the crude product showed two peaks on GLC [ECL 23.9 (90%), and 24.2 (5%)]. The last ECL was the same as that of authentic methyl 9,12-epithiostearate.

5. Preparation of methyl 9,12-epithiostearate from an unsaturated mercapto ester

Methyl 9,12-epithiostearate (reaction with sodium hydrogen sulphide)

Methyl 9-mesyloxyoctadec-cis-12-enoate (496 mg, 1.6 mmole), prepared from the hydroxy ester in the usual way, was added to a solution of sodium hydrogen sulphide (300 mg, 5.36 mmole) in dry dimethylformamide (5 ml). The reaction mixture was swirled and kept at room temperature for 30 hr. After addition of water, the solution was extracted with ether (30 ml) and the aqueous organic layer was acidified with sulphuric acid (2M, 2ml) and re-extracted with ether (30 ml). The combined ethereal extracts, after drying over anhydrous sodium sulphate, yielded a product (421 mg) which showed three bands (A, B, and C) on TLC (PE25).

Band A (70-75%) of ECL 24.2 was considered to be methyl 9,12-epithiostearate on the basis of the following evidence.

(i) A sample of this component, submitted to Mozingo hydrogenolysis, gave a product which behaved like an authentic sample of methyl stearate (ECL 18.0).

(ii) The NMR and IR spectra along with TLC and GLC, showed

that the product was unchanged after attempted reactions with acetyl chloride and with trifluoroacetic anhydride.

(iii) After an attempted desulphurisation reaction with methyl iodide and dry acetone (a characteristic reaction for desulphurisation of 1,2-epithio compounds [121]) the product was shown to be the unchanged starting material both on TLC (PE25) and GLC (ECL 24.2).

(iv) A sample of band A was analysed. Found: C, 69.35; H, 10.96: Calc. for $C_{19}H_{36}O_2S$: C, 69.56; H, 10.98.

(v) The NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, $-COOCH_3$), 6.45-6.95 (m, 2H, $-CH(S)CH_2CH_2CH-$), 7.82 (t, 2H, $-CH_2COOCH_3$), 8.65 (br.s, 26H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

(vi) The mass spectrum contained a molecular ion peak at m/e 328. Other details have been reported in the Discussion.

(vii) The structure was finally confirmed by comparison with a synthetic sample of methyl 9,12-epithiostearate prepared by a completely independent route.

Band B (15-20%) gave no peak on GLC and may contain disulphides formed during the reaction.

Band C (5%) showed the same TLC behaviour as starting material.

Experiments carried out to discover the mechanism of the formation of methyl 9,12-epithiostearate during the reaction of methyl 9-mesyloxyoctadec-cis-12-enoate with sodium hydrogen sulphide in dimethylformamide at room temperature

(i) Reaction carried out in the presence of an antioxidant[191]

Methyl 9-mesyloxyoctadec-cis-12-enoate (280 mg, 0.72 mmole) was added to a solution of sodium hydrogen sulphide (150 mg, 2.7 mmole) and of butylated hydroxytoluene (BHT, 40 mg) in dry

dimethylformamide (10 ml). The reaction mixture was kept at room temperature for 24 hours. After the addition of water, the solution was extracted with ether (30 ml), the aqueous organic layer was acidified with hydrochloric acid (2M) and re-extracted with ether (30 ml). The combined ethereal extracts, after drying yielded a product (261 mg) a portion of which (150 mg) was separated into four bands (X, A, B, and C) on TLC (PE25).

Band X (30 mg, 20%) looked like a non-lipid material when viewed under UV lamp and behaved like an authentic sample of butylated hydroxy-toluene (BHT) on TLC (PE25).

Band A (75 mg, 50%) showed two peaks on GLC [ECL 24.2 (65%) and 23.6 (30%)]. After acetylation (65 mg) the product was successfully separated into two subfractions: A_1 (24 mg, ECL 24.2) and A_2 (30 mg, ECL 26.1). A_1 showed the sample TLC and GLC behaviour as methyl 9,12-epithiostearate, A_2 was methyl 9-acetylmercapto-octadec-cis-12-enoate on the basis of its IR, NMR and MS. The infrared spectrum (liquid film) showed a diagnostic absorption band at 1685 cm^{-1} ($SCOCH_3$). The NMR spectrum (CCl_4, 100 MHz) contained signals at 4.72 (m, 2H, -CH=CH-), 6.40 (s, 3H, $-COOCH_3$), 6.44-6.62 (m, 1H, $-CHSCOCH_3-$), 7.80-8.04 (m, 4H, $-CH_2CH=CHCH_2-$), 8.22 (m, 2H, $-CH_2CH(SCOCH_3)-$), 8.70 (br.s, 18H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

Band B (9 mg, 10%) gave no peak on GLC and may contain disulphides formed during the reaction.

Band C was possibly the starting material.

(ii) Reaction in the presence of a neutralising agent

Methyl 9-mesyloxyoctadec-cis-12-enoate (200 mg, 0.51 mmole) was added to a solution of sodium hydrogen sulphide (120 mg, 2.1 mmole)

and calcium carbonate (770 mg, 7.7 mmole) in dry dimethylformamide (5 ml). The reaction mixture was kept at room temperature for 24 hours. After the addition of water, the ether extract contained 158 mg [ECL 23.6 (25%) and 24.2 (70%)]. A second extract isolated after acidification (hydrochloric acid, 2M) of the aqueous organic layer gave additional material [10 mg, ECL 23.6 (5%) and 24.2 (90%)]. Both extracts showed a peak of ECL 19.4 (ca 5%) which was possibly the dienoate resulting from the elimination of mesyloxy ester.

(iii) Reaction in an inert atmosphere

Methyl 9-mesyloxyoctadec-cis-12-enoate (350 mg, 0.90 mmole) and sodium hydrogen sulphide (180 mg, 3.2 mmole) in dry dimethylformamide (5 ml) were kept at room temperature under nitrogen for 24 hours. The reaction mixture was cooled at 0^{o} (ice bath) and then diluted with water. Material extracted before and after the acidification (hydrochloric acid, 2M) of the aqueous organic layer amounted to 280 mg and 38 mg respectively. The larger extract showed two major peaks of ECL 23.6 (20%) and 24.2 (73%) and the smaller extract had ECL of 23.6 (4%) and 24.2 (90%). Both extracts showed a peak of ECL 19.4 (ca 5%) which possibly resulted from the dienoates produced by an elimination reaction.

(iv) Reaction followed by immediate acetylation

Methyl 9-mesyloxyoctadec-cis-12-enoate (225 mg, 0.58 mmole) and sodium hydrogen sulphide (120 mg, 2.1 mmole) in dry dimethylformamide (5 ml) were kept at room temperature for 24 hours. After cooling at 0^{o}, water was added and aqueous organic layer was extracted with ether [2 x 20 ml, the second portion after the acidification (2M, hydrochloric acid)]. The two ethereal extracts were combined together and dried (182 mg). The crude ester (112 mg)

was purified by prep TLC (PE25). The major band A yielded 81 mg. Band A (65 mg) was immediately acetylated and then separated into two subfractions A_1 (25 mg, ECL 24.2) and A_2 (80 mg, ECL 26.1). The subfraction A_2 was methyl 9-acetylmercapto-octadec-cis-12-enoate on the basis of its GLC (ECL 26.1), IR and NMR. The infrared spectrum (liquid film) showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and the NMR spectrum (CCl_4, 100 MHz) contained signals at 4.72 (m, 2H, -CH=CH-) and at 7.74τ (s, 3H, $-SCOCH_3$) along with other usual signals.

(v) Reaction of methyl 9-acetylmercapto-octadec-cis-12-enoate with methanolic sulphuric acid

Methyl 9-acetylmercapto-octadec-cis-12-enoate (23 mg, ECL 26.1) was kept at room temperature overnight with methanolic sulphuric acid (1M, 2 ml). Next day, the product (19 mg) was extracted with ether and examined by GLC [ECL 24.2 (40%) and 26.1 (60%)]. The peak of ECL 26.1 disappeared completely and the peak at 24.2 remained when the ester was refluxed with methanolic sulphuric acid (1M, 1 ml) for 2 hours.

Attempts to prepare methyl 9,12-epithiostearate by other procedures

(i) From 9,12-dihydroxystearic acid

A mixture of 9,12-dihydroxystearic acid (98 mg, 0.3 mmole) and phosphorous pentasulphide[189] (200 mg, 0.7 mmole) were refluxed in dry benzene (4 ml) for 4 hours. The product was recovered by partition between ether and water and esterified (boron trifluoride/methanol). The ester (50 mg), examined by TLC (PE25, PE50, E100), behaved like a highly polar or polymeric compound which did not move from the base line of the TLC plate.

The experiment was repeated using a solvent in which phosphorous pentasulphide was soluble. To a mixture of 9,12-dihydroxystearic acid (50 mg, 0.17 mmole), phosphorous pentasulphide (100 mg, 0.45 mmole) in dry pyridine (4 ml) was added slowly. During the addition an exothermic reaction occurred. When the phosphorous pentasulphide was completely dissolved in pyridine, the mixture was refluxed at 140° (oil bath) for 4 hours. After acidification (hydrochloric acid, 2M, 5 ml), the aqueous organic layer was extracted with ether (2 x 20 ml) and the product again behaved like a highly polar or polymeric compound.

(ii) Methyl 9,12-epoxystearate (40 mg, 0.13 mmole) and phosphorous pentasulphide[190] (90 mg, 0.37 mmole) were refluxed in dry benzene for 4 hours. After addition of water, the product was extracted with ether (2 x 20 ml) and then examined by TLC (PE25). The product showed no difference between the starting material on both TLC (PE25) and GLC (ECL 20.62).

6. Preparation of methyl 9-mercapto-octadec-cis-12-enoate

Methyl 9-acetylmercapto-octadec-cis-12-enoate (reaction with potassium thiolacetate)

Methyl 9-mesyloxyoctadec-cis-12-enoate (270 mg, 0.70 mmole) and potassium thiolacetate (300 mg, 1.6 mmole) in dimethylformamide (10 ml) were heated at 100°C for 3 hours. After cooling, water was added and the reaction mixture was extracted with ether [2 x 30 ml, the second extraction was made after acidifying the aqueous

organic layer with hydrochloric acid (2M)]. The product (282 mg) was purified by prep TLC (PE25) to yield a product (147 mg) which was considered to be methyl 9-acetylmercapto-octadec-cis-12-enoate on the basis of its GLC, IR, and NMR. GLC showed a single peak of ECL 26.1. The infrared spectrum (liquid film) contained a characteristic strong absorption band at 1685 cm^{-1} ($SCOCH_3$). The NMR spectrum (CCl_4, 100 MHz) showed signals at 4.72 (m, 2H, -CH=CH-), 6.40 (s, 3H, $-COOCH_3$), 6.44-6.62 (m, 1H, $-CHSCOCH_3$-), 7.74 (s, 3H, $-SCOCH_3$), 7.78 (t, 2H, $-CH_2COOCH_3$), 7.80-8.04 (m, 4H, $-CH_2CH=CHCH_2-$), 8.22 (m, 2H, $-CH_2CH(SCOCH_3)-$), 8.70 (br.s, 18H, $-(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-).

Reaction of methyl 9-acetylmercapto-octadec-cis-12-enoate with methanolic sodium methoxide

Methyl 9-acetylmercapto-octadec-cis-12-enoate (109 mg, 0.3 mmole) was refluxed with methanolic sodium methoxide (0.2M, 5 ml) in the presence of Zn/Hg for 2 hr. The reaction mixture was cooled to 0^o and then diluted with degassed water. The aqueous layer was made acidic with hydrochloric acid (1M) and extracted with ether (2 x 20 ml). The isolated product (87 mg) appeared as a single spot on TLC (PE25). After purification by prep TLC (PE24) the product (71 mg) was considered to be methyl 9-mercapto-octadec-cis-12-enoate on the basis of its NMR. GLC showed two peaks of ECL* 24.2 (75%) and 23.6 (20%). The NMR spectrum (CCl_4, 100 MHz) contained signals at 4.72 (m, 2H, -CH=CH), 6.42 (s, 3H, $-COOCH_3$), 7.32 (m, 1H, -CHSH-), 7.78 (t, 2H, $-CH_2COOCH_3$), 7.80-8.00 (m, 4H, $-CH_2CH=CHCH_2-$), 8.52 (m, 2H, $-CH_2CHSH-$),** 8.70 (br.s, ca 19H, $-(CH_2)_n-$), and 9.12τ (t, 3H, $-CH_3CH_2-$).

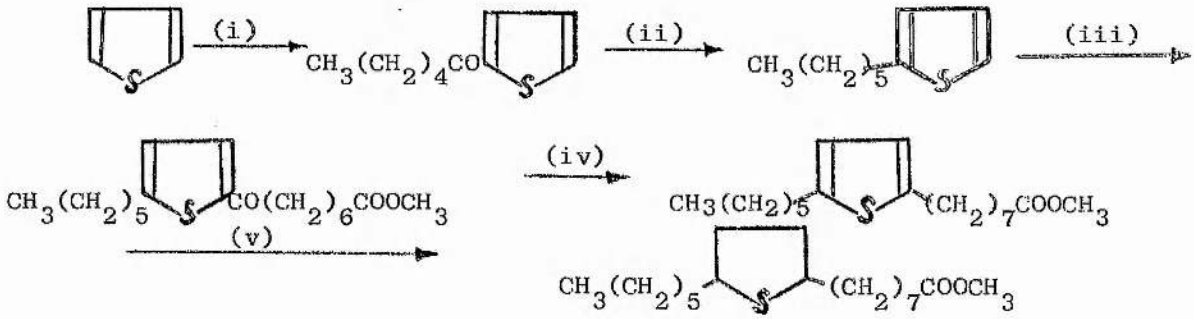
* This peak was formed possibly during GLC.

** The single proton of -CHSH was always hidden by the polymethylene broad singlet at 8.70 τ

Reaction of methyl 9-acetylmercapto-octadec-cis-12-enoate with methanolic sulphuric acid

Methyl 9-acetylmercapto-octadec-cis-12-enoate (30 mg, ECL 26.1) was added to a solution of methanolic sulphuric acid (1M, 1 ml) and kept at room temperature overnight. Next day, the product (26 mg) was extracted with ether and examined by GLC (peaks of ECL 24.2 (40%) and 26.1 (60%)]. The peak of ECL 26.1 disappeared completely with the formation of only peak of ECL 24.2, when the ester was refluxed with methanolic sulphuric acid (1M, 1 ml) for 3 hours.

7. Synthesis of methyl 9,12-epithiostearate



(i) $CH_3(CH_2)_4COCl;SnCl_4$ (ii) $N_2H_4;(HOCH_2CH_2)_2O,KOH$

(iii) $ClCO(CH_2)_6COOCH_3;SnCl_4$ (iv) $N_2H_4;(HOCH_2CH_2)_2O,KOH$

(v) Pd/H_2

Hexanoyl chloride[192,193]

Freshly distilled thionyl chloride (65.6 g, 0.55 mmole) was placed into a three-necked flask fitted with a reflux condenser and a separating funnel containing hexanoic acid (30 g, 0.26 mmole). The flask was heated gently on a water bath and the hexanoic acid

was added dropwise during 30-40 minutes. When all the acid had been added, the mixture was refluxed for half an hour and then distilled (25 g, 73%, b.p. 150-153°/760 mm; lit.[194] b.p. 151-53°/760 mm).

2-Hexanoylthiophen[195]

A solution of hexanoyl chloride (20 g, 0.15 mmole) and thiophen (14 g, 0.17 mmole) in dry benzene (170 ml) was cooled to 0° and freshly distilled stannic chloride (44 g, 0.17 mole) was added in small portions during 30 minutes at 0-5°. After removal of the cooling bath the mixture was stirred at room temperature for 1 hour and hydrochloric acid (1M, 110 ml) was added with cooling. The benzene layer was washed with water and evaporated. Distillation of the residue gave 2-hexanoylthiophen (22 g, 82%, b.p. 116-118°/2 mm; lit.[196] b.p. 117-119°/1 mm, ECL 15.6 at 170°).
Found: C, 66.08; H, 8.03 : Calc. for $C_{10}H_{14}OS$: C, 65.92; H, 7.70: TLC showed a single spot of low Rf. The infrared (liquid film) spectrum showed a diagnostic absorption band at 1660 cm^{-1} (CO) and the NMR spectrum (CCl_4, 100 MHz) contained signals at 2.45 (m, 2H, -COC(S)=CHCH=CH-), 2.96 (dd, 1H, -COC(S)=CHCH=CH-), 7.20 (t, 2H, $-CH_2CO-$), 8.30 (m, 2H, $-CH_2CH_2CO-$), 8.64 (br.s, 4H, $-(CH_2)_2-$) and 9.08τ (t, 3H, CH_3CH_2-).

The mass spectrum contained a molecular ion peak at m/e 182. Other details have already been discussed in the Discussion.

2-Hexylthiophen

2-Hexanoylthiophen (15 g, 0.082 mmole) was stirred with hydrazine hydrate (17 g, 0.35 mmole) in diethylene glycol (200 ml) at 120° for ½ hr. Potassium hydroxide (15 g, 0.27 mmole) in hot diethylene glycol (100 ml) was added, and the mixture was stirred at 150-155° for 1 hour. The water formed during the reaction and

the excess of hydrazine hydrate were allowed to distil off during 1 hour until the internal temperature reached 190°. Stirring was continued at this temperature for a further 3 hours. The solution was poured onto ice (300 g), acidified by hydrochloric acid (2M, 85 ml) and extracted with chloroform (2 x 30 ml). Evaporation of the washed (water, 30 ml) and dried (anhydrous sodium sulphate) solution gave on distillation 2-hexylthiophen (12.6 g, 90%, b.p. 58-59°/0.5 mm, ECL 9.6 at 110°). Found: C, 71.29; H, 9.54: Calc. for $C_{10}H_{16}S$, C, 71.44; H, 9.52. TLC (PE15) showed a single spot. The NMR spectrum (CCl_4, 100 MHz) contained signals at 3.02 (m, 1H, $-CH_2C(S)=CHCH=\underline{C}H-$), 3.20 (m, 1H, $-CH_2C(S)=\underline{C}HCH=CH-$), 3.32 (dd, 1H, $-CH_2C(S)=CHC\underline{H}=CH-$), 7.20 (t, 2H, $-\underline{C}H_2C(S)=CHCH=CH-$), 8.40 (m, 2H, $-\underline{C}H_2CH_2C(S)=CHCH=CH-$), 8.66 (br.s, 4H, $-(\underline{C}H_2)_2-$) and 9.10τ (t, 3H, $\underline{C}H_3CH_2-$).

The mass spectrum showed a molecular ion peak at m/e 168. Other details have already been given in the Discussion.

7-Carbomethoxyheptanoyl chloride

7-Carbomethoxyheptanoic acid (7 g, 0.037 mmole) and freshly distilled thionyl chloride (15 g, 0.12 mmole) were reacted in the usual way. The crude product (8 g) was used for the next step.

Methyl 8-(2'-5'-hexylthienyl)-8-oxo-octanoate

Hexylthiophen (1.2 g, 7.1 mmole) and 7-carbomethoxyheptanoyl chloride (4 g, 20.00 mmole) in dry benzene (15 ml) were treated with stannic chloride in the usual way. The crude product (5.5 g) showed several spots on TLC (PE25) and a portion of this (3.5 g) was purified by prep TLC (PE25). The major band (1 g) was considered to be methyl 8-(2'-5'-hexylthienyl)-8-oxo-octanoate on the basis of its IR, NMR and mass spectrum. The infrared spectrum (liquid film)

showed a strong diagnostic absorption band at 1660 cm^{-1} (CO) and the NMR spectrum (CCl_4, 100 MHz) contained signals at 2.58 (d, J=4.5, 1H, $-CH_2C(S)=CHCH=C-CO-$), 3.30 (d, J=4.5, 1H, $-CH_2C(S)=CHCH=C-CO$), 6.42 (s, 3H, $-COOCH_3$), 7.20 (t, 2H, $-CH_2C(S)=CHCH=C-COCH_2$), 7.26 (t, 2H, $-CH_2C(S)=CHCH=C-COCH_2-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.38 (m, 2H, $-CH_2C(S)=CHCH=C-COCH_2CH_2-$), 8.65 (br.s, 14H, $-(CH_2)_n-$) and 9.10τ (t, 3H, CH_3CH_2-).

The mass spectrum had a molecular ion peak at m/e 338. Other details have already been given in the Discussion.

Methyl 8-(2'-5'-hexylthienyl)octanoate

The keto ester (0.55 g, 1.5 mmole) was reduced with hydrazine hydrate (2 g, 0.04 mmole) in diethylene glycol (7 ml) in the usual way and the product (0.53 g) was purified by prep TLC (PE25). The major band (360 mg, ECL 23.5) was considered to be methyl 8-(2'-5'-hexylthienyl)octanoate on the basis of its IR and NMR spectra. The infrared spectrum (liquid film) showed no absorption band at 1660 cm^{-1} (CO). The NMR spectrum (CCl_4, 100 MHz) contained signals at 3.58 (s, 2H, $-CH_2C(S)=CHCH=CCH_2-$), 6.42 (s, 3H, $-COOCH_3$), 7.30 (t, 4H, $-CH_2C(S)=CHCH=CCH_2-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.44 (m, 4H, $-CH_2CH_2C(S)=CHCH=CCH_2CH_2-$), 8.66 (br.s, 14H, $(CH_2)_n-$) and 9.11τ (t, 3H, CH_3CH_2-).

Its mass spectrum had a molecular ion peak at m/e 324 and another fragment at 293 (M-31). Other details have already been discussed in the Discussion.

Methyl 9,12-epithiostearate

Palladium charcoal (10%, 500 mg) was reduced by shaking with hydrogen at atmospheric pressure. To the reduced catalyst suspended in methanol (5 ml) was added one drop of concentrated sulphuric acid

and methyl 8-(2'-5'-hexylthienyl)octanoate (180 mg) dissolved in methanol (2 ml). After 24 hours the product showed a new peak of ECL 24.2 (5%). Additional catalyst was added at intervals of 24 hours up to 96 hours. The final product showed three bands (A, B, and C) on TLC (PE25).

Band A (10%) showed no peaks on GLC and was not examined further.

Band B (30%) behaved like an authentic sample of methylstearate both on TLC and GLC (ECL 18.0).

Band C (50%) had an ECL of 24.2. It was considered to be methyl 9,12-epithiostearate on the basis of its NMR and mass spectrum. The NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, $-COOCH_3$), 6.45-6.95 (br.m., 2H, $-CH(S)CH_2CH_2CHCH_2-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.65 (br.s, 26H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

The mass spectrum had a molecular ion peak at m/e 328. Other fragmentation details have already been given in the Discussion.

The synthetic methyl 9,12-epithiostearate and the product formed by the reaction of methyl 9-mesyloxyoctadec-cis-12-enoate and sodium hydrogen sulphide behaved identically on GLC (ECL 24.2) and gave similar NMR spectra.

8. Preparation of methyl 9(10)-mercaptostearate by radical addition

Starting materials: Oleic acid (98%) was obtained from olive oil acids by urea fractionation as described by Schlenk and Holman[197]. The acid was esterified with methanolic hydrogen chloride (2M). Thiolacetic acid was purified by distillation (b.p. 87°). Ditertiary-butyl peroxide (technical grade) was used as supplied.

Methyl 9(10)-acetylmercaptostearate from oleic acid

Oleic acid (2.08 g, 7.34 mmole), thiol acetic acid (1.14 g, 15.0 mmole) and ditertiary-butylperoxide (15 mg) were heated under nitrogen at 60°C for 2 days (8-9 hours daily); Additional ditertiary-butyl-peroxide (150 mg) was then added and heating was continued 1 day longer. The reaction mixture was diluted (30 ml) and successively washed with water (2 x 20 ml), aqueous sodium hydroxide (0.1M, 2 x 10 ml), sodium sulphite (1M, 2 x 10 ml) and finally with water until the water washings were free of acid. The ether phase was evaporated to yield a pale yellow oil (2.3 g). The infrared spectrum (liquid film) of the crude acid contained absorption bands at 1700 (COOH) and 1680 cm^{-1} ($SCOCH_3$). An attempt to purify the crude mercapto acid by urea complex method was unsatisfactory.

The crude acetylmercapto acid (2.0 g) was deacetylated by refluxing with potassium hydroxide (1.8 g) in aqueous methanol (1:1, 18 ml). The reaction mixture after acidification (2M, hydrochloric acid) was extracted with ether (2 x 20 ml) and then evaporated to give a pale yellow oil (1.62 g). The infrared spectrum (liquid film) of the crude oil contained no absorption band at 1685 cm^{-1} ($SCOCH_3$). After esterification (boron trifluoride/methanol) the product (1.13 g) showed two less polar major bands A and B on TLC (PE15) along with several polar diffuse bands.

Band A (53%) showed a major peak of ECL 23.3 (80%). It was considered to be methyl 9(10)-mercaptostearate on the basis of its NMR spectrum (CCl_4, 60 MHz) which showed signals at 6.40 (s, 3H, $COOCH_3$), 7.25-7.65 (m, 1H, -CHSH-), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.70 (br.s, ca 26H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-). After acetylation and purification by prep TLC (PE15), the product showed a single peak on GLC (ECL 26.0) and its infrared spectrum (liquid film) contained a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) whilst its NMR spectrum (60 MHz, CCl_4) showed a three-proton singlet at 7.72τ ($SCOCH_3$). Hydrogenolysis of band A gave a product which behaved like an authentic sample of methyl stearate (ECL 18.0).

Band B (20%)showed no peak on GLC and was possibly the dimer of methyl 9(10)-mercaptostearate.

Methyl 9(10)-acetylmercaptostearate from methyl oleate

Methyl oleate (960 mg, 3.24 mmole), thiol acetic acid (530 mg, 7.5 mmole) and ditertiary-butylperoxide (100 mg) were heated under nitrogen at 60°C for 3 days (8-9 hours daily); additional ditertiary-butylperoxide (200 mg) was then added and the reaction was continued for another 2 days. Worked up as previously described, the product was a pale yellow oil (970 mg) which showed a less polar major spot along with several diffuse spots of higher polarity. The infrared spectrum (liquid film) of the crude ester contained strong absorption bands at 1685 ($SCOCH_3$) and 1735 cm^{-1} ($COOCH_3$). A sample of crude ester (143 mg), purified by prep TLC (PE20), gave a major band (64 mg, 45%) which was considered to be methyl 9(10)-acetyl-mercaptostearate on the basis of its GLC, IR and NMR. GLC showed a single peak of ECL 26.0 and its infrared spectrum (liquid film)

contained a strong absorption band at 1685 cm^{-1} ($SCOCH_3$). The NMR spectrum (CCl_4, 60 MHz), compared with that of methyl oleate, showed an additional signal at 7.72 (s, 3H, $SCOCH_3$) with a complete absence of olefinic protons (~4.5τ).

Methyl 9(10)-mercaptostearate

Methyl 9(10)-acetylmercaptostearate (510 mg) was hydrolysed by refluxing with potassium hydroxide (450 mg) in aqueous methanol (1:1, 5 ml) for 2 hours. The crude acid (355 mg) which contained no infrared absorption band at 1685 cm^{-1} ($SCOCH_3$) was esterified (boron trifluoride/methanol) and the ester was separated into two major components (A and B) along with several polar minor bands.

Band A (57%, ECL 23.3) was considered to be methyl 9(10)-mercapto-stearate on the basis of the following evidence:

(i) After acetylation this material (ECL 26.0) contained a diagnostic strong infrared absorption band at 1685 cm^{-1} ($SCOCH_3$).

(ii) Hydrogenolysis (Mozingo) of Band A gave a product which behaved like an authentic sample of methyl stearate on GLC (ECL 18.0).

(iii) The NMR spectrum (CCl_4, 60 MHz) contained no olefinic signals (4.5τ). It had the same signals as those listed for band A in the previous experiment.

Band B (19%) showed no peak on GLC, appeared to be unchanged after treatment with sodium borohydride in methanol, and gave methyl stearate (ECL 18.0) on hydrogenolysis. It is considered to be the dimer of methyl 9(10)-mercaptostearate.

9. Preparation of methyl 9,12-epithiostearate from hydroxy mercapto esters

(i) From methyl ricinoleate

Methyl 9(10)-acetylmercapto-12-hydroxystearate

Methyl ricinoleate (2 g, 6.4 mmole), thiol acetic acid (1 g, 13.25 mmole) and ditertiary-butylperoxide (150 mg) were heated at 60°C under nitrogen for 3 days (8-9 hours daily); additional ditertiary-butylperoxide (200 mg) was then added and heating was continued for another 3 days longer. The crude product was a pale yellow oil (2.26 g) which contained strong infrared absorption bands at 1685 ($SCOCH_3$), 1735 ($COOCH_3$) and 3500 cm^{-1} (OH). Purification of the crude ester by the urea complex method was unsatisfactory, but was achieved in 61% yield by prep TLC (PE25). The product was considered to be methyl 9(10)-acetylmercapto-12-hydroxystearate on the basis of its IR and NMR spectra. The infrared spectrum (liquid film) showed absorption bands at 1685 ($SCOCH_3$), 1735 ($COOCH_3$) and 3500 cm^{-1} (OH). The NMR spectrum (CCl_4, 60 MHz) contained signals at 6.40 (S, 3H, $COOCH_3$), 6.35 (m, *ca 3H, $-CH(SCOCH_3)-$, $-CHOH-$), 7.72 (s, 3H, $-CH(SCOCH_3)-$), 8.25 (m, 2H, $-CH(OH)CH_2CH(SCOCH_3)-$), 8.68 (br.s, ca 22H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

Its mass spectrum showed a peak at m/e 389 (M^++1) along with other peaks which are already reported in the Discussion.

Methyl 12-hydroxy-9(10)-mercaptostearate

Methyl 9(10)-acetylmercapto-12-hydroxystearate (60 mg, 0.15 mmole) was deacetylated by refluxing with methanolic sodium methoxide (0.17M, 2 ml) for 2 hours to give the hydroxymercapto ester (52 mg).

* The integral for the multiplet at 6.35-6.65τ was equivalent to three protons.

The infrared spectrum (liquid film) of the ester showed strong characteristic absorption band at 3500 cm^{-1} (OH) and its bis-trifluoroacetyl derivative contained absorption bands at 1700 ($SCOCF_3$), 1735 ($COOCH_3$) and 1770 cm^{-1} ($OCOCF_3$). The GLC of the trifluoroacetylated product showed peaks of ECL 22.7, 23.0 and 23.5. The NMR spectrum (CCl_4, 100 MHz) of the diacetyl derivative showed signals at 6.40 (s, 3H, $-COOCH_3$), 6.40-6.80 (m, 2H, $-CHSCOCH_3-$, $-CHOCOCH_3-$), 7.72 (s, 3H, $-SCOCH_3$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.04 (s, 3H, $OCOCH_3$), 8.28 (m, 2H, $-CH(OCOCH_3)CH_2CH(SCOCH_3)-$), 8.70 (br.s, ca 22H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

The mass spectrum of methyl 12-hydroxy-9(10)-mercapto ester contained peaks at m/e 328 ($M^+ - 18$) and 297 $[M^+ - (18 + 31)]$. Other details have already been given in the Discussion.

Methyl 9,12-epithiostearate

Methyl 9(10)-acetylmercapto-12-hydroxystearate (113 mg, 0.3 mmole) was refluxed with methanolic sulphuric acid (2.5M, 10 ml) for 2 hours. The resulting product was diluted with water and then extracted with ether (2 x 20 ml). Evaporation of the solvent afforded a product (93 mg) which showed three bands (A, B, and C) on TLC (PE25).

Band A (20-25%, ECL 24.2 with an inflection at 24.1). Its NMR spectrum (CCl_4, 100 MHz) contained a broad signal at 6.50-6.95τ (2H). Signals for $SCOCH_3$ and for olefinic protons were absent. Hydrogenolysis gave methyl stearate (ECL 18.0). The mass spectrum showed a molecular ion peak at m/e 328 and had its base peak at m/e 171. Other details of fragmentation have already been discussed in the Discussion.

Band B (50-60%)which showed strong infrared absorption band at 3500 cm^{-1} (OH) was converted to its trifluoroacetyl derivative (ECL 22.7,

23.0 and 23.5). The infrared spectrum (liquid film) contained absorption bands at 1700 ($SCOCF_3$), 1735 ($COOCH_3$) and 1770 cm^{-1} ($OCOCF_3$). Hydrolysis (methanolic sodium methoxide) and subsequent reaction with trifluoroacetic anhydride gave the same bis TFA derivative (ECL 22.7, 23.0 and 23.5). Its acetyl derivative showed infrared absorption bands at 1685 ($SCOCH_3$) and 1735 cm^{-1} ($COOCH_3$) and had NMR (CCl_4, 100 MHz) signals at 6.40 (s, 3H, $COOCH_3$), 6.40-6.80 (m, 2H, $-CHSCOCH_3-$, $-CHOCOCH_3$), 7.72 (s, 3H, $SCOCH_3$), 8.04 (s, 3H, $OCOCH_3$), 8.28 (m, 2H, $-CH(OCOCH_3)CH_2CH(SCOCH_3)-$), 8.70 (br.s, ca 22H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-). Hydrolysis (methanolic sodium methoxide) and deacetylation gave a product identical with the starting material. The mass spectrum of band B had major peaks at m/e 328 (M^+ - 18) and m/e 297 (328-31). Other details have already been discussed fully in the Discussion.

Band C (15%) was accompanied by several other minor diffuse bands and was not examined further.

(ii) From methyl 9-hydroxy-cis-12-enoate

Methyl 12(13)-acetylmercapto-9-hydroxystearate

Methyl 9-hydroxyoctadec-cis-12-enoate (635 mg, 2 mmole), thiol acetic acid (500 mg, 6.6 mmole) and ditertiary-butylperoxide (200 mg) were reacted as already described. The crude ester (750 mg) was purified by prep TLC (PE25). The major band (464 mg, 62%) was proved to be methyl 12(13)-acetylmercapto-9-hydroxystearate on the basis of its IR and NMR spectra. Its infrared spectrum (liquid film) contained absorption bands at 1685 ($SCOCH_3$), 1735 ($COOCH_3$) and 3500 cm^{-1} (OH) and the NMR spectrum (CCl_4, 60 MHz) showed signals

at 6.40 (s, 3H, $-COOC\underline{H}_3$), 6.40-6.80 (m, 2H, $-C\underline{H}(SCOCH_3)-$, $-C\underline{H}(OH)-$), 7.72 (s, 3H, $SCOC\underline{H}_3$), 7.82 (t, *3H, $-C\underline{H}_2COOCH_3$) , 8.65 (br.s, 24H, $-(C\underline{H}_2)_n-$) and 9.12τ (t, 3H, $C\underline{H}_3CH_2-$).

Its mass spectrum had peaks at m/e 389 ($M^+ + 1$), 371 (389-18) and 328 (371-43). Other details have already been discussed in the Discussion.

Methyl 12(13)-mercapto-9-hydroxystearate

Methyl 12(13)-acetylmercapto-9-hydroxystearate (55 mg) was deacetylated by refluxing with methanolic sodium methoxide (0.17M, 2 ml) to give the hydroxymercapto ester (48 mg). The infrared spectrum (liquid film) of the product showed a strong absorption band at 3500 cm^{-1} (OH) and complete absence of absorption at 1685 cm^{-1} ($SCOCH_3$). After reacting with trifluoroacetic anhydride, the product gave three peaks on GLC (ECL 22.7, 23.0 and 23.3). Its infrared spectrum (liquid film) contained absorption bands at 1700 ($SCOCF_3$), 1735 ($COOCH_3$) and 1770 cm^{-1} ($OCOCF_3$). The acetylated derivative showed absorption bands at 1685 ($SCOCH_3$) and 1735 cm^{-1} ($COOCH_3$). Its NMR spectrum (CCl_4, 100 MHz) contained two three-proton singlets at 7.72 ($SCOC\underline{H}_3$) and 8.05 ($OCOC\underline{H}_3$) along with other usual signals. The NMR spectrum (CCl_4, 100 MHz) of the trifluoroacetylated derivative showed all usual signals except two three-proton signals at 7.72 ($SCOC\underline{H}_3$) and 8.05τ ($OCOC\underline{H}_3$).

Methyl 9,12-epithiostearate

Methyl 12(13)-acetylmercapto-9-hydroxystearate (137 mg, 0.44 mmole) was refluxed with methanolic sulphuric acid (2.5M, 12 ml)

* The signal of single proton $-CH(O\underline{H})-$ was possibly hidden by the triplet at 7.82τ. Its integral was equivalent to three protons.

for 2 hours, and the resulting ester was diluted with water and then extracted with ether (2 x 20 ml). After evaporation of solvent, the product (102 mg) showed three bands (A, B and C) on TLC (PE25).

Band A (20-25%, ECL 24.2 with an inflection at ECL 24.1). Its NMR spectrum (CCl_4, 100 MHz) contained a broad signal at 6.50-6.96τ (2H). Signals for $SCOCH_3$ and two olefinic protons were absent. Hydrogenolysis gave methyl stearate (ECL 18.0). The mass spectrum showed a molecular ion peak at m/e 328 and had its base peak at m/e 171.

Band B (50-60%). The infrared spectrum (liquid film) of this band contained strong absorption band at 3500 cm^{-1} (OH). After trifluoroacetylation the product had three peaks on GLC (ECL 22.7, 23.0 and 23.3) and its infrared spectrum (liquid film) showed absorption bands at 1700 ($SCOCF_3$), 1735 ($COOCH_3$) and 1770 cm^{-1} ($OCOCF_3$). Hydrolysis followed by reaction with trifluoroacetic anhydride gave a bis TFA derivative (ECL 22.7, 23.0, and 23.5) identical with the starting material. After acetylation the NMR spectrum (CCl_4, 100 MHz) showed signals at 6.40 (s, 3H, $COOCH_3$), 6.40-6.80 (m, 2H, $-CH(SCOCH_3)$ and $-CH(OCOCH_3)-$), 7.72 (s, 3H, $SCOCH_3$), 7.80 (t, 2H, $-CH_2COOCH_3$), 8.04 (s, 3H, $OCOCH_3$), 8.70 (br.s, 24H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-). Hydrolysis (methanolic sodium methoxide) and re-acetylation again gave a product identical with the starting material. The mass spectrum of band B had major peaks at m/e 328 (M-18) and m/e 297 [M-(18+31)].

Band C (16%) was accompanied by several other minor bands and was not examined further.

10. Preparation of αβ-epithio esters from the corresponding epoxides

Methyl cis-9,10-epithiostearate[121]

A solution of methyl cis-9,10-epoxystearate (255 mg, 0.82 mmole) in dioxan (5 ml) was slowly added to a solution of thiourea (70 mg, 0.92 mmole) in water (2 ml) and conc sulphuric acid (50 mg; sp. gr. 1.84) with stirring, and the mixture kept at room temperature overnight. Aqueous sodium carbonate (0.07M, 3 ml) was then added to the reaction mixture which was maintained at 40° for 1 hour. Acidification (1M, H_2SO_4) and then ether extraction (2 x 20 ml) gave a product (243 mg) which showed two spots on TLC (PE20), one was unreacted methyl 9,10-epoxystearate and the other was less polar than the starting material. The less polar band (60 mg, 30%) was considered to be methyl cis-9,10-epithiostearate. It showed a major peak on GLC (ECL 18.5 with tailing which might be due to decomposition on the column). The NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, $-COOCH_3$), 7.20 (m, 2H, $-\underset{\diagdown S \diagup}{CH}-CH-$(cis)), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.70 (br.s, 26H, $-(CH_2)_n-$) and 9.11τ (t, 3H, CH_3CH_2-).

The mass spectrum showed major peaks at m/e 296 (M-32), 265 (296-31) and 264 (296-32). Full details have already been given in the Discussion.

Cis-9,10-epithiostearic acid

A solution of 9,10-epoxystearic acid (1 g, 3.25 mmole) in dioxan (5 ml) was reacted with a solution of thiourea (250 mg, 3.30 mmole) in water (5 ml) and conc sulphuric acid (165 mg; sp. gr. 1.84) as already described. The product was a colourless solid (852 mg) a portion of which (170 mg) was esterified (boron

trifluoride/methanol) and then purified by prep TLC (PE25). The non polar band (63 mg) which behaved like methyl cis-9,10-epithiostearate on both TLC and GLC was reacted with acetyl chloride and then purified by TLC (72 mg). The product showed strong infrared absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 60 MHz) contained signals at 6.05-6.58 (m, 2H, $-CH_2\underline{CH}(Cl)\underline{CH}(SCOCH_3)-$), 6.40 (s, 3H, $COOCH_3$), 7.70 (s, 3H, $-CH(SCOCH_3)-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.72 (br.s, 26H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

Methyl trans-9,10-epithiostearate

Methyl trans-9,10-epoxystearate (160 mg, 0.51 mmole, prepared from methyl elaidate by reaction with m-chloro perbenzoic acid) was treated with thiourea solution [50 mg, 0.64 mmole in water (2 ml) and conc sulphuric acid (40 mg)] as already described. The crude colourless solid (177 mg), purified by prep TLC (PE25), gave a product (45 mg) which was considered to be methyl trans-9,10-epithiostearate on the basis of its GLC, NMR and MS. GLC showed major peaks of ECL 18.6 (40%) and 24.0 (55%). The peak of ECL 18.6 was accompanied by some tailing peaks which might be due to on-column decomposition. Its NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (t, 3H, $-COOCH_3$), 7.54 (m, 2H, $-\underline{CH}-\underline{CH}-$(trans)), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.72 (br.s, 26H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

The mass spectrum showed a molecular ion peak at m/e 328 with other fragments at 294 (M-34) and 263 (294-31). Other details have already been given in the Discussion.

Desulphurisation with methyl iodide[121,166]

A mixture of methyl trans-9,10-epithiostearate (25 mg, 0.08 mmole) and methyl iodide (125 mg, 0.09 mmole) in dry acetone (125 mg,

2.6 mmole) was heated under reflux for 1 hour during which time the mixture turned dark brown and an obnoxious smelling gas was evolved. After cooling, iodine formed during the reaction was removed by washing with sodium thiosulphate (1M) and the solution was extracted with ether (2 x 10 ml). The ethereal extracts were washed once with dilute sodium thiosulphate solution (1M, 5 ml) and twice with water (10 ml), dried (Na_2SO_4) and then evaporated to afford a pale yellow solid (31 mg) which showed three bands (A-C) on TLC (PE25).

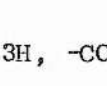
Band A (30%, ECL 18.6) contained a characteristic absorption band at 965 cm^{-1} (-CH=CH-) in its infrared spectrum (liquid film).

Band B (50%) behaved like unreacted methyl trans-9,10-epithiostearate both on TLC and GLC.

Band C looked like a non-lipid material when viewed under the UV lamp and was possibly the $(CH_3)_3S^+I^-$ formed during the reaction. This fraction had an obnoxious smell.

Methyl 12,13-epithio-octadec-cis-9-enoate

Methyl 12,13-epoxyoctadec-cis-9-enoate (85 mg, 0.28 mmole) was reacted with a solution of thiourea (40 mg) in water (2 ml) and conc sulphuric acid (40 mg, sp. gr. 1.84) as previously described. The crude ester (92 mg) was purified by prep TLC (PE25) to give a product which was considered to be methyl 12,13-epithio-octadec-cis-9-enoate (39 mg) on the basis of its GLC and NMR. It showed two peaks on GLC [19.6 (70%) with tailing (which was possibly methyl octadecadienoate formed by the extrusion of sulphur) and 26.2 (30%)] whereas methyl 12,13-epoxyoctadec-cis-9-enoate gave a single peak of ECL 24.3 on GLC. Its NMR spectrum (CCl_4, 100 MHz) contained signals at 4.58 (m, 2H,

-CH=CH-), 6.42 (s, 3H, $-COOCH_3$), 7.18 (m, 2H, ), 7.30 (m, 2H,  $CH_2CH=CH-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.00 (m, 2H, $-CH=CHCH_2-$), 8.67 (br.s, 18H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

Reaction with methanolic sulphuric acid

Methyl 12,13-epithio-octadec-cis-9-enoate (18 mg) was refluxed with methanolic sulphuric acid (1M, 2 ml) for 3 hours. The product (19 mg) showed several bands on TLC (PE25). The major band (7 mg, ECL 21.8) examined by NMR (CCl_4, 100 MHz, microcell) contained an additional three-proton singlet at 6.68τ (OCH_3) in addition to the other usual signals and was thought to be a methoxy mercapto alkenoate.

11. Preparation of αβ-epithio esters from the corresponding alkenoates

Methyl threo-9,10-dithiocyanato-octadecanoates[161]

A vigorously stirred suspension of freshly prepared lead thiocyanate[198,199] (825 mg, 2.6 mmole; dried over P_2O_5 in the dark in vacuo) in anhydrous* acetic acid (15 ml) was treated with dry bromine** (270 mg) and left until colourless. To the stirred suspension was then added methyl oleate (500 mg, 1.7 mmole) and

* Acetic acid was made anhydrous by refluxing with acetic anhydride (5% by weight) for 4 hours.

** Bromine was dried by shaking with an equal weight of conc sulphuric acid.

stirring was continued for 16 hours. Next day the mixture was filtered and poured into water (50 ml), extracted with ether (2 x 20 ml), washed until free of acid and dried. The product (625 mg) was purified by prep TLC (PE25) and the major band (97 mg, from 120 mg of the product) showed a characteristic infrared absorption band at 2080 cm^{-1} (-C≡N stretching).

(i) Action of sodium sulphide on the threo dithiocyanato ester

The dithiocyanato ester (253 mg, 0.72 mmole) in ethanol (5 ml) was refluxed for 30 minutes with a solution of sodium sulphide nonahydrate (1 g, 4.1 mmole) in ethanol (10 ml). The cooled and diluted mixture was acidified with hydrochloric acid (2M). The product (222 mg), obtained by ether extraction, behaved like an acid on analytical TLC (PE25). A portion (100 mg) was esterified (boron trifluoride/methanol) and purified by prep TLC (PE25). The major band (53 mg) behaved like methyl cis-9,10-epithiostearate on both TLC and GLC (ECL 18.5 thought to be 18:1 formed in the column).

A similar result was also obtained when anhydrous* sodium sulphide was used.

(ii) Action of methanolic sodium methoxide on the dithiocyanato ester

Methyl threo-9,10-dithiocyanato-octadecanoate (48 mg, 0.11 mmole) was refluxed with methanolic sodium methoxide (0.1M, 3 ml) for 30 minutes. The solution was cooled, diluted with water, acidified with hydrochloric acid (1M) and extracted with ether (2 x 10 ml). The crude ester (37 mg) showed a single spot of low Rf. Purification by prep TLC afforded a product (28 mg) which was

* Anhydrous sodium sulphide was obtained by keeping sodium sulphide nonahydrate at 110° overnight.

considered to be methyl cis-9,10-epithiostearate from its TLC and GLC (ECL 18.5, thought to be 18:1) behaviour.

(iii) Action of lithium borohydride on the threo dithiocyanato ester

Methyl threo 9,10-dithiocyanato-octadecanoate (47 mg, 0.13 mmole, purified by prep TLC) in tetrahydrofuran (1 ml) was added to a stirred mixture[200,201] of sodium borohydride (100 mg) and anhydrous* lithium chloride (100 mg) in tetrahydrofuran (5 ml). The reaction mixture was heated under reflux for 30 minutes. Excess hydride was destroyed by the cautious addition of dilute acetic acid until evolution of gas ceased. The product (40 mg) was separated into two bands (A and B) by TLC (PE25).

Band A (70%) behaved like an authentic sample of methyl cis-9,10-epithiostearate on both TLC and GLC (ECL 18.5, with tailing, possibly due to on-column decomposition) and this view was confirmed from its NMR spectrum (CCl_4, 100 MHz) which contained a characteristic signal at 7.20τ (m, 2H,  -(cis)) in addition to other usual signals.

Trifluoroacetylation of this material furnished a product which contained the characteristic absorption bands at 1780 ($OCOCF_3$), 1735 ($COOCH_3$) and 1700 cm^{-1} ($SCOCF_3$). A similar result was also obtained when an authentic sample of methyl cis-9,10-epithiostearate was trifluoroacetylated. The mass spectrum of band A showed major peaks at m/e 296 (M-32), 265 (296-31) and 264 (296-32). The details of other fragments have already been discussed in the Discussion.

Band B (25%) which showed several peaks on GLC was not examined further.

*Anhydrous lithium chloride was obtained by keeping lithium chloride monohydrate at 150° for 3 hours and then under vacuo over phosphorous pentoxide overnight.

12. Preparation of methyl 10,13-epithio-12-mercaptostearate

Methyl 10,13-epithio-12-mercaptostearate

Methyl threo 12,13-dimesyloxyoctadec-cis-9-enoate (378 mg, 0.8 mmole; prepared from the corresponding threo-dihydroxy ester in the usual manner) and sodium hydrogen sulphide (420 mg, 7.5 mmole) in dimethylformamide (10 ml) were kept at room temperature for 24 hours. The mixture was diluted with water, acidifed with hydrochloric acid (2M) and then extracted with ether (2 x 20 ml). On TLC (PE25) the crude product (265 mg) showed two major bands (A and B) at low Rf along with several diffuse polar bands.

Band A (30%). This fraction was believed to be methyl 10,13-epithio-12-mercaptostearate and showed a late running peak of ECL ca 30 on GLC. Its infrared and NMR spectra were examined after acetylation and trifluoroacetylation. The infrared spectrum (liquid film) of the acetylated and trifluoroacetylated products showed absorption bands at 1685 ($SCOCH_3$) and 1700 cm^{-1} ($SCOCF_3$) respectively. The NMR spectrum (CCl_4, 100 MHz) of band A contained signals at 6.40 (s, 3H, $-COOCH_3$), 6.40-6.80 (m, 2H, $-HC(S)CH(SH)CH_2CH-$), 6.80-7.20 (m, 1H, $-HC(S)CH(SH)CH_2CH-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 7.90 (m, 2H, $-HC(S)CH(SH)CH_2CH-$), 8.70 (br.s, 21H, $-(CH_2)_n-$) and 9.08τ (t, 3H, CH_3CH_2-). Its acetyl derivative had similar signals with an additional three-proton singlet at 7.72τ ($SCOCH_3$). The mass spectrum of methyl 10,13-epithio-12-mercaptostearate and its acetyl derivative are reported in the Discussion.

Band B (20%) showed no peak on GLC and was not examined further. It was possibly the dimer.

13. Preparation of methyl 9,10-dimercaptostearate from methyl 9,10-dihydroxystearate

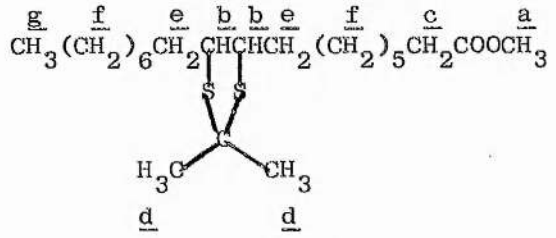
Methyl 9,10-dimercaptostearate from methyl erythro-9,10-dihydroxystearate

Methyl erythro-9,10-dimesyloxystearate (588 mg, 1.2 mmole), prepared from the erythro-dihydroxy ester in the usual way, was reacted with a solution of sodium hydrogen sulphide (600 mg, 10.7 mmole) in dimethylformamide (15 ml) at room temperature for 24 hours. The product (492 mg) showed two major bands A and B along with some minor polar diffuse bands on TLC (PE25).

Band A (50%) showed a late-running peak of ECL ca 32 on GLC. It was considered to be methyl 9,10-dimercaptostearate on the basis of the following evidence.

(i) The infrared spectrum (liquid film) of band A showed a diagnostic absorption band at 2580 cm^{-1} (-SH) and its NMR spectrum (CCl_4, 100 MHz) contained signals at 5.40 (s, 3H, $COOCH_3$), 7.16 (m, 2H, $-CH(SH)CH(SH)-$), 8.40, 8.48 (m, 4H, $-CH_2CH(SH)CH(SH)CH_2-$), *8.70 (br.s, ca 29H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

(ii) The isopropylidene derivative of methyl 9,10-dimercaptostearate, prepared[63] by reacting with acetone in the presence of a few drops of conc sulphuric acid, showed the following signals in its NMR spectrum (CCl_4, 100 MHz):



6.40 (s, 3H, a), 6.58-6.64 (m, 2H, b), 7.78 (t, 2H, c), 8.27 (s, 6,

* The two protons of -CH(SH)CH(SH)- were hidden by the polymethylene broad singlet at 8.70

d), 8.42 (m, 4H, e), 8.72 (br.s, 22H, f), and 9.12τ (t, 3H, g).

(iii) After acetylation it showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) contained signals at 6.24-6.42 (m, 2H, $-CH(SCOCH_3)CH(SCOCH_3)$), 7.72 (s, 6H, $CH(SCOCH_3)CH(SCOCH_3)$), 7.80 (t, 2H, $-CH_2COOCH_3$), 8.40-8.60 (m, 4H, $-CH_2CH(SCOCH_3)CH(SCOCH_3)CH_2-$), 8.75 (br.s, 22H, $-(CH_2)_n-$) and 9.13τ (t, 3H, CH_3CH_2-).

(iv) The mass spectrum of isopropylidene derivative of methyl 9,10-dimercaptostearate had a molecular ion peak at m/e 402 and a fragment at 387 (M-15). Full details of its fragmentation pattern have already been discussed in the Discussion.

Band B (ca 20%) which showed no peak on GLC was proved to be dimer of methyl 9,10-dimercaptostearate. Samples of band A and B were reduced by lithium aluminium hydride and the products from each were reacted with trifluoroacetic anhydride. The trifluoroacetylated derivatives showed similar behaviour on GLC (ECL 16.5) and both contained infrared absorption bands at 1770 ($COCF_3$) and 1700 ($SCOCF_3$) cm^{-1}.

Methyl 9,10-diacetylmercaptostearate from methyl erythro 9,10-dimesyloxystearate

Methyl erythro 9,10-dimesyloxystearate (120 mg, 0.25 mmole) was heated with potassium thiolacetate (250 mg, 2.2 mmole) in dimethylformamide (5 ml) at 100°C for 4 hours. The product (122 mg) showed two major bands A and B along with some minor polar diffuse bands on TLC (PE25).

Band A (16%) Its infrared spectrum (liquid film) contained absorption bands at 1685 cm^{-1} ($SCOCH_3$) and the NMR spectrum (CCl_4, 100 MHz) showed a two-proton olefinic signal at 4.60τ and a three-proton signal

at 7.72 ($SCOCH_3$) along with other usual signals. It was thought to be the elimination product produced by the reaction with potassium thiolacetate.

Band B (55%) was considered to be methyl 9,10-diacetylmercaptostearate. Its infrared spectrum (liquid film) showed an absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) contained signals at 6.25-6.40 (m, 2H, $-CH(SCOCH_3)CH(SCOCH_3)-$), 6.40 (s, 3H, $-COOCH_3$), 7.72 (s, 6H, $-CH(SCOCH_3)CH(SCOCH_3)-$), 7.80 (t, 2H, CH_2COOCH_3), 8.40-8.60 (m, 4H, $-CH_2CH(SCOCH_3)CH(SCOCH_3)CH_2-$), 8.75 (br.s, 22H, $-(CH_2)_n-$) and 9.13τ (t, 3H, CH_3CH_2-).

Methyl 9,10-dimercaptostearate from methyl threo-9,10-dimesyloxystearate

Methyl threo-9,10-dimesyloxystearate (500 mg, 1.03 mmole) was reacted with a solution of sodium hydrogen sulphide (600 mg, 10.7 mmole) in dimethylformamide (10 ml) at room temperature for 24 hours. The product (393 mg) showed two bands (A and B) along with several polar diffuse bands on TLC (PE25).

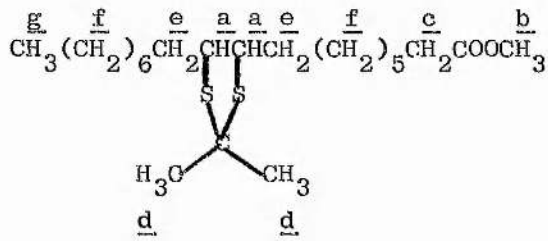
Band A (50%) which showed a late-running peak of ECL ca 32 on GLC was considered to be methyl 9,10-dimercaptostearate on the basis of the following evidence.

(i) The infrared spectrum (liquid film) of band A showed a diagnostic absorption band at 2580 cm^{-1} (SH-) and its NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, $COOCH_3$), 7.16 (m, 2H, $-CH(SH)CH(SH)-$), 8.40-8.50 (m, 4H, $-CH_2CH(SH)CH(SH)CH_2-$), *8.70 (br.s, 24H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

(ii) The isopropylidene derivative of methyl 9,10-dimercaptostearate

* The two protons of $-CH(SH)CH(SH)-$ were hidden by the broad singlet at 8.70τ.

prepared by reacting with acetone in the presence of a few drops of conc sulphuric acid at room temperature overnight and was examined by NMR. Its NMR spectrum (CCl_4, 100 MHz) showed the following signals:



6.20-6.44 (m, 2H, a), 6.40 (s, 3H, b), 7.78 (t, 2H, c), 8.82 (s, 6H, d), 8.42 (m, 4H, e), 8.70 (br.s, 22H, f) and 9.12τ (t, 3H, g).

(iii) After acetylation the product showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) in its IR spectrum (liquid film) and its NMR spectrum contained signals at 6.20-6.42 (m, 2H, $-CH(SCOCH_3)CH(SCOCH_3)-$), 7.72 (s, 6H, $-CH(SCOCH_3)CH(SCOCH_3)-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.42-8.52 (m, 4H, $-CH_2CH(SCOCH_3)CH(SCOCH_3)CH_2-$), 8.72 (br.s, 22H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

(iv) The mass spectrum of the isopropylidene derivative of methyl 9,10-dimercaptostearate had a molecular ion peak at m/e 402 and a fragment at 387 (M-15). The details have already been discussed in the Discussion.

Band B (20%) showed no peak on GLC and was possibly the dimer of methyl 9,10-dimercaptostearate.

Methyl 9,10-diacetylmercaptostearate from methyl threo-9,10-dimesyloxystearate

Methyl threo-9,10-dimesyloxystearate (125 mg, 0.26 mmole) was reacted with potassium thiolacetate (250 mg, 2.2 mmole) in dimethylformamide (5 ml) in the usual way. The product (117 mg) showed two major bands A and B along with some minor polar diffuse bands on TLC (PE25).

Band A (15%) The infrared spectrum (liquid film) of this fraction showed an absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) contained a two-proton olefinic signal at 4.65τ and a three-proton singlet at 7.70τ ($SCOCH_3$) along with other usual signals. It was thought to be the elimination product produced during the reaction with potassium thiolacetate.

Band B (50%) was considered to be methyl 9,10-diacetalmercaptostearate. The infrared spectrum (liquid film) showed strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) contained a six-proton signal at 7.72τ along with other usual signals.

An attempt to prepare methyl 9,10-dimercaptostearate by another route

Trithiocarbonato-acids from cis-9,10-epoxy acids

To a solution of potassium hydroxide (1 g, 0.018 mmole) in dry methanol (20 ml) was added carbon disulphide (1.5 g, 0.02 mmole) and cis-9,10-epoxystearic acid (500 mg, 1.7 mmole) and the mixture was kept at room temperature for 2 weeks. Thereafter, the deep yellow solution was poured into water (50 ml), acidified with dilute hydrochloric acid (2M) and extracted with ether. The ethereal solution was washed with water until free of acid, dried (Na_2SO_4), and evaporated to yield threo-9,10-(thiocarbonyldithio)-stearic acid (565 mg). The infrared spectrum (Nujol mull) of the crude product showed a strong absorption band at 1070 (C=S) cm^{-1}

(i) Action of lithium aluminium hydride on threo-thiocarbonyldithio-stearic acid

The crude threo-thiocarbonyldithio acid (250 mg, 0.52 mmole) in dry ether (5 ml) was heated under reflux with lithium aluminium hydride for 2 hours. The resulting product (182 mg) showed a major band along

with several diffuse polar bands. An attempt was made to acetylate the major band (60 mg) but the product contained no absorption band at 1685 cm^{-1}. The product was a complex mixture of several bands (TLC) which was not examined further.

(ii) Action of lithium borohydride on threo-thiocarbonyldithiostearic acid

To sodium borohydride (330 mg, 9.0 mmole), and anhydrous lithium chloride (330 mg, 9.0 mmole) in tetrahydrofuran (10 ml) was added threo-9,10-thiocarbonyldithio acid (360 mg, 0.9 mmole) in tetrahydrofuran (5 ml) drop by drop (efficient stirring) over 5 minutes, then the mixture was refluxed for 30 minutes. Excess borohydride was destroyed by cautious addition of dilute acetic acid (2M) until no further effervescence occurred, and the product was extracted with ether. The ethereal solution was washed with ether until free of acid, dried (Na_2SO_4) and evaporated to afford a pale yellow oil (312 mg). A portion of this (142 mg) was esterified (boron trifluoride/methanol) and examined by TLC (PE25). The crude ester was a complex mixture of several bands and a single compound could not be isolated even after acetylation.

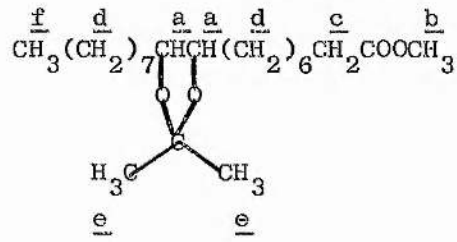
(iii)
Action of lithium borohydride or methyl threo-thiocarbonyldithiostearate

Methyl threo-thiocarbonyldithio ester (140 mg, 0.34 mmole, purified by prep TLC) was reduced with lithium borohydride (prepared in situ by the action of sodium borohydride and anhydrous lithium chloride) in tetrahydrofuran (5 ml) in the similar manner. The product (131 mg) showed several bands on TLC (PE25) which could not be isolated for further studies.

14. Preparation of methyl erythro- and threo-9,10-isopropylidene-dioxystearate

Methyl erythro-9,10-isopropylidenedioxystearate[155,156]

Methyl erythro-9,10-dihydroxystearate (100 mg, 0.3 mmole) was refluxed with dry acetone (15 ml) in the presence of anhydrous* copper sulphate (500 mg) for 1 hour. The crude product (112 mg) was purified by prep TLC (PE40, 85 mg, ECL 22.1). Its NMR spectrum (CCl_4, 100 MHz) contained the following signals:

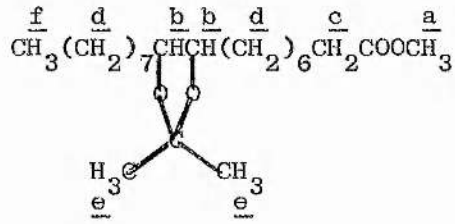


6.06-6.20 (m, 2H, a), 6.40 (s, 3H, b), 7.78 (t, 2H, c), **8.67 (br.s, ca 32H, d and e) and 9.12τ (t, 3H, f).

The mass spectrum had a molecular ion peak at m/e 370 and a characteristic fragment at m/e 355 (370-15). Details of the other fragments have already been given in the Discussion.

Methyl threo-9,10-isopropylidenedioxystearate

Methyl threo-9,10-dihydroxystearate (85 mg) was reacted with acetone (15 ml) in a similar manner. The purified (prep TLC) product (71 mg, ECL 22.2) contained the following signals in its NMR spectrum (CCl_4, 100 MHz):



*Blue copper sulphate ($CuSO_4,5H_2O$) was made anhydrous by keeping it overnight at 250°C

**The methyl protons of isopropylidenedioxy ester were hidden by the polymethylene broad singlet at 8.67τ. The integral for this broad signal was equivalent to ca 32 protons.

6.40 (s, 3H, a), 6.54-6.66 (m, 2H, b), 7.78 (t, 2H, c), 8.73 (br.s, ca 32H, d and e) and 9.12τ (t, 3H, f).

The mass spectrum showed molecular ion peak at m/e 370 and a characteristic fragment at m/e 355 (370-15). Details of other fragments have already been reported in the Discussion.

15. Preparation of methyl 9,12- and 10,12-epidithiostearates from the corresponding dihydroxy esters

Methyl 9,12- and 10,12-dihydroxystearates [160,202]

Pure methyl ricinoleate (5.5 g, 0.018 mmole) was shaken with mercuric acetate (6.5 g, 0.023 mmole) in water (50 ml) and tetrahydrofuran (75 ml) for 4 days and then reduced with sodium borohydride (1.5 g) to give a product which was examined on GLC as TMS-ethers [ECL 20.5 (21%), 20.9 (17%), 23.2 (8%) and 23.4 (54%)]. Prep TLC (PE70) on the product (100 mg) gave four bands A-D.

Band A (15 mg) behaved like methyl 9,12-epoxystearate both on TLC and GLC (ECL 20.9).

Band B (18 mg) had the same TLC and GLC (ECL 20.5 as TMS ether) behaviour as methyl ricinoleate.

Band C (7 mg) which appeared as a double spot, was considered to be methyl 10,12-dihydroxystearate (ECL 23.2 as TMS ether) and

Band D (50 mg) to be methyl 9,12-dihydroxystearate (ECL 23.4 as TMS-ether) on the basis of the following reaction:

The two methyl dihydroxystearates (5 mg each) were refluxed separately with methanolic sulphuric acid (2M, 1 ml) for 8 hours.

The double spotted band C remained unchanged. There was no trace of band D which was replaced by a less polar spot spectroscopically identical with methyl 9,12-epoxystearate.

Methyl 10,12- and 9,12-dihydroxystearate were separated by prep TLC (PE70).

Methyl 9,12-dimesyloxystearate

Methyl 9,12-dihydroxystearate [250 mg, 0.80 mmole, purified by prep TLC (PE70)] in pyridine (6 ml) was stirred with methanesulphonyl chloride (2 g, 1.7 mmole) for 5 hours at room temperature. Recovery as described previously afforded a product (298 mg) which showed two bands on TLC (PE70).

Band A (18%) which showed the presence of a three-proton singlet at 7.02τ may be the chloro mesyl ester although chloride was not detected after sodium fusion.

Band B (70%) showed a six-proton singlet at 7.08τ along with other usual signals and was considered to be methyl 9,12-dimesyloxystearate.

Methyl 9,12-epithiostearate

Methyl 9,12-dimesyloxystearate [145 mg, 0.4 mmole; purified by prep TLC (PE70)] was reacted overnight with a solution of sodium hydrogen sulphide (150 mg, 2.7 mmole) in dimethylformamide (5 ml). The product (112 mg) showed a single spot on TLC (PE50) and was considered to be methyl 9,12-epithiostearate (ECL 24.2) on the basis of the following evidence:

(i) A sample of this component was purified by double development on prep TLC (PE25) and analysed. Found: C, 69.58; H, 11.53: calc. for $C_{19}H_{36}O_2S$: C, 69.56; H, 10.98.

(ii) The NMR spectrum (CCl_4, 100 MHz) showed signals at 6.40

(s, 3H, $-COOCH_3$), 6.45-6.95 (m, 2H, $-CH(S)CH_2CH_2CH-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.65 (br.s, 26H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

(iii) The mass spectrum contained a molecular ion peak at m/e 328 and a base peak at 171 which is a characteristic fragment of methyl 9,12-epithiostearate. Details have already been given in the Discussion.

Methyl 9,12-diacetylmercaptostearate

Methyl 9,12-dimesyloxystearate (150 mg, 0.31 mmole) was heated at 100°C with potassium thiolacetate (300 mg) in dimethylformamide (6 ml) for 3 hours. The product (165 mg) showed a major band (112 mg, probably methyl 9,12-diacetylmercaptostearate). The infrared spectrum (liquid film) of this component showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and the NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, $-COOCH_3$), 6.44-6.78 (m, 2H, $-CH(SCOCH_3)CH_2CH_2CH(SCOCH_3)$), 7.72 (s, 6H, $-CH(SCOCH_3)CH_2CH_2CH(SCOCH_3)-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.40-8.46 (m, 4H, $-CH(SCOCH_3)CH_2CH_2CH(SCOCH_3)$), 8.71 (br.s, 22H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

Methyl 9,12-epidithiostearate

Methyl 9,12-diacetylmercaptostearate (81 mg, 0.21 mmole) was heated under reflux with a methanolic solution of sodium methoxide (0.2M, 5 ml) for 30 minutes. After cooling, the reaction mixture was diluted with water, acidified with hydrochloric acid (0.1M) and then extracted with ether (2 x 20 ml). The ether extracts were dried (Na_2SO_4) and evaporated to afford a product (69 mg) which showed a single spot (ECL ca 30) on TLC (PE25). This was considered to be methyl 9,12-epidithiostearate. The NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, $-COOCH_3$), 7.16-7.28 (m, 2H,

$-CH(S)CH_2CH_2CH(S)-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 7.96-8.20 (m, 4H, $-CH(S)CH_2CH_2CH(S)-$), 8.68 (br.s, 22H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

The mass spectrum had a molecular ion peak at m/e 360 along with other fragments which have already been given in the Discussion.

This compound remained unchanged on treatment with ethanolic iodine solution (1M) overnight.

Methyl 9,12-dimercaptostearate

Methyl 9,12-diacetylmercaptostearate (85 mg, 0.19 mmole) was stirred with methanolic hydrochloric acid (5M, 5 ml) in the presence of Zn/Hg (100 mg) for 40 minutes[26]. The reaction mixture was diluted with degassed water and the aqueous organic layer was extracted with ether (2 x 20 ml). The ethereal extracts were washed with degassed water until free of acid, dried and then evaporated. The product (69 mg) showed two bands on TLC (PE25).

Band A (52%) showed a single late-running peak on GLC (ECL ca30) and was considered to be methyl 9,12-dimercaptostearate on the basis of its IR, NMR and MS spectra. The infrared spectrum (liquid film) showed a characteristic absorption band at 2580 cm^{-1} (SH) and its NMR spectrum contained signals at 6.42 (s, 3H, $-COOCH_3$), 7.33 (m, 2H, $-CH(SH)CH_2CH_2CH(SH)-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.30-8.50 (m, 4H, $-CH(SH)CH_2CH_2CH(SH)-$),* 8.70 (br.s, ca 24H, $-(CH_2)_n-$) and 9.12τ (t, 3H, $-CH_3CH_2-$).

The mass spectrum had a molecular ion peak at m/e 362 along with other fragments which have been reported in the Discussion.

* The two protons of $-CH(SH)CH_2CH_2CH(SH)-$ were hidden by the broad singlet at 8.70τ.

A sample of band A (26 mg) after acetylation (31 mg) with acetic anhydride/anhydrous sodium acetate showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) in its infrared spectrum (liquid film). Its NMR spectrum (CCl_4, 100 MHz) contained a diagnostic six-proton signal at 7.72τ ($SCOCH_3$) along with other usual signals.

Band B (20%) showed a three-proton singlet in its NMR spectrum (CCl_4, 100 MHz) at 7.72τ ($SCOCH_3$) and was thought to be partially acetylated product.

Deacetylation of the reacetylated methyl 9,12-dimercaptostearate

Regenerated methyl 9,12-diacetylmercaptostearate (22 mg, 0.044 mmole) was refluxed with methanolic hydrochloric acid (5M, 5 ml) for 30 minutes. The product (19 mg), isolated using degassed water during work-up, showed a single spot on TLC (PE25). Its infrared spectrum (liquid film) contained a characteristic absorption band at 2585 cm^{-1} (SH). The mass spectrum showed a molecular ion peak at m/e 362 with other fragments which have already been reported in the Discussion.

Action of methyl 9,12-dimercaptostearate with iodine

Methyl 9,12-dimercaptostearate (12 mg) was kept at room temperature overnight with an ethanolic solution of iodine (0.1M, 2 ml). Next day, the excess of iodine was removed by washing with sodium thiosulphate and the product which showed a single spot on TLC (PE25) was purified and examined by MS. The mass spectrum had a molecular ion peak at m/e 360 along with other characteristic fragments. On treatment with acetic anhydride(anhydrous sodium acetate)the compound was recovered unchanged.

Action of lithium aluminium hydride on methyl 9,12-epidithiostearate

Methyl 9,12-epidithiostearate (15 mg, 0.031 mmole) was reduced with lithium aluminium hydride (50 mg, 1.2 mmole) in dry ether(5 ml). The reaction mixture was cooled to 0^{o} and the excess of hydride was destroyed by the cautious addition of wet ether and then of water. The aqueous organic layer was acidified with hydrochloric acid (0.1M) after cooling (ice bath). The product (15 mg) was acetylated by refluxing with acetic anhydride (2 ml) in the presence of a few crystals of anhydrous sodium acetate. After purification by prep TLC (PE25) the acetylated product (10 mg) was examined by IR and NMR. The infrared spectrum (liquid film) showed diagnostic strong absorption bands at 1685 ($SCOCH_3$) and 1750 cm^{-1} ($OCOCH_3$). The NMR spectrum (CCl_4, 100 MHz, microcell) contained signals at 6.03 (t, 2H, $C\underline{H}_2OCOCH_3$), 6.42-6.68 (m, 2H, $-C\underline{H}(SCOCH_3)CH_2CH_2C\underline{H}(SCOCH_3)-$), 7.72 (s, 6H, $-CH(SCOC\underline{H}_3)CH_2CH_2CH(SCOC\underline{H}_3)-$), 8.06 (s, 3H, $-CH_2OCOC\underline{H}_3$), 8.38-8.44 (m, 4H, $-CH(SCOCH_3)C\underline{H}_2C\underline{H}_2CH(SCOCH_3)-$), 8.70 (br.s, 22H, $-(C\underline{H}_2)_n-$), and 9.12τ (t, 3H, $C\underline{H}_3CH_2-$).

Methyl 10,12-epidithiostearate

Methyl 10,12-dimesyloxystearate [85 mg, 0.017 mmole, prepared from the dihydroxy ester in the usual way and then purified by prep TLC (PE50)] was reacted with a solution of sodium hydrogen sulphide (110 mg, 2 mmole) in dimethylformamide (5 ml) at room temperature overnight. Working up as described previously afforded a product (68 mg) which showed a single spot on TLC (PE25). The purified product (prep TLC) had an ECL of 24.8 on GLC and its NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, $-COOC\underline{H}_3$), 6.44-6.68 (m, 2H, $-C\underline{H}(S)CH_2C\underline{H}(S)-$), 7.78 (t, 2H, $-C\underline{H}_2COOCH_3$), 8.42 (m, 2H, $-CH(S)C\underline{H}_2CH(S)-$), 8.68 (br.s, 24H, $-(C\underline{H}_2)_n-$), and 9.12τ (t, 3H,

CH_3CH_2-). The mass spectrum had a molecular ion peak at m/e 360. Full details have already been reported in the Discussion.

Methyl 10,12-diacetylmercaptostearate

Methyl 10,12-dimesyloxystearate (102 mg, 0.21 mmole) prepared from the dihydroxy ester and then purified by prep TLC, was heated at 100°C with potassium thiolacetate (200 mg, 1.7 mmole) in dimethylformamide (5 ml) for 3 hours. The product (103 mg) showed a major component (55%) on TLC (PE25). This component was probably methyl 10,12-diacetylmercaptostearate. The infrared spectrum (liquid film) showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, -$COOCH_3$), 6.42-6.66 (m, 2H, -$CH(SCOCH_3)CH_2CH(SCOCH_3)$-), 7.76 (s, 6H, -$CH(SCOCH_3)CH_2CH(SCOCH_3)$-), 7.78 (t, 2H, -$CH_2COOCH_3$), 8.34 (m, 2H, -$CH(SCOCH_3)CH_2CH(SCOCH_3)$-), 8.70 (br.s, 24H, -$(CH_2)_n$-), and 9.12τ (t, 3H, CH_3CH_2-).

Methyl 10,12-epidithiostearate

Methyl 10,12-diacetylmercaptostearate (31 mg, 0.09 mmole) was refluxed with a methanolic solution of sodium methoxide (0.2M, 3 ml) in the presence of Zn/Hg (50 mg) for 1 hour. The reaction mixture was cooled to 0°, diluted with degassed water, acidified (just acidic) with hydrochloric acid (0.1M) and then extracted with ether. The product (27 mg) showed a single spot on TLC (PE25) and was considered to be methyl 10,12-epidithiostearate. Its NMR spectrum (CCl_4, 100 MHz) contained signals at 6.40 (s, 3H, -$COOCH_3$), 7.00 (m, 2H, -$CH(S)CH_2CH(S)$-), 7.78 (t, 2H, -CH_2COOCH_3), 8.26 (m, 2H, -$CH(S)CH_2CH(S)$-), 8.68 (br.s, 24H, -$(CH_2)_n$-) and 9.12τ (t, 3H, CH_3CH_2-). The mass spectrum had a molecular ion peak at m/e 360 and other fragments which are detailed in the Discussion.

Deacetylation of methyl 10,12-diacetylmercaptostearate by methanolic hydrochloric acid

Methyl 10,12-diacetylmercaptostearate (15 mg) was heated under reflux with a methanolic solution of hydrochloric acid (5M, 2 ml) for 1 hour. The product (9 mg), after purification by prep TLC (PE25), was examined by mass spectrometry. The spectrum showed a molecular ion peak at m/e 362. Full details have been reported in the Discussion.

The isopropylidene derivative of methyl 10,12-dimercaptostearate had signals at 6.90-7.25 (m, 2H, $(CH_3)_2(CHS)_2-CH_2-$) and 8.42τ (s, 6H, $(CH_3)_2-(CHS)_2CH_2-$) in its NMR spectrum (CCl_4, microcell, 100 MHz). Its mass spectrum had a molecular ion peak at m/e 402 with other fragment at 387 (M-15). Full details have already been given in the Discussion.

An attempt to prepare methyl 10,12-dimercaptostearate by another route

Methyl 9(10),12-diacetylmercaptostearate (radical addition of thiol-acetic acid to methyl 12-acetylmercapto oleate)

Methyl 12-acetylmercapto oleate (560 mg, 1.3 mmole), thiolacetic acid (1 ml) and ditertiary-butylperoxide (100 mg) were heated at 60-70° under nitrogen (8-9 hours daily) for 3-4 days. The product (721 mg) showed a major spot on TLC (PE25). Purification afforded 635 mg. Its NMR spectrum (CCl_4, 100 MHz) contained signals at 6.42 (s, 3H, $-COOCH_3$), 6.45-6.60 (m, 2H, $-CH(SCOCH_3)(CH_2)_{2(1)}CH(SCOCH_3)-$), 7.74 (s, 6H, $-CH(SCOCH_3(CH_2)_{2(1)}CH(SCOCH_3)-$), 7.78 (t, 2H, $-CH_2COOCH_3$), 8.24-8.58 (m, ca 8H, $-CH_2CH(SCOCH_3)(CH_2)_{2(1)}CH(SCOCH_3)CH_2-$), 8.71 (br.s, ca 18H, $-(CH_2)_n-$), and 9.12τ (t, 3H, $-CH_3CH_2-$).

Deacetylation of methyl 9(10),12-diacetylmercaptostearate

Methyl 9(10),12-diacetylmercaptostearate (219 mg) was refluxed with a methanolic solution of hydrochloric acid (5M, 15 ml) in the presence of Zn/Hg (200 mg). The reaction mixture was cooled to 0^{o}, diluted with degassed water and then extracted with ether (2 x 20 ml). The ethereal extracts were washed with water until free of acid, dried and evaporated. The product (180 mg) showed a major spot on TLC (PE25). Its mass spectrum showed a molecular ion peak at m/e 362 with other fragments at 360, and 328.

An attempt to prepare the isopropylidene derivative of methyl 9(10),12-dimercaptostearate

Methyl 9(10),12-dimercaptostearate (50 mg) was treated with acetone (5 ml) at room temperature overnight in the presence of a few drops of conc sulphuric acid. The product (47 mg) after the purification by prep TLC (PE25) was examined by NMR (CCl_4, 100 MHz). But the spectrum contained no signals around 8.30τ $[(CH_3)_2C=]$.

16. Preparation of 2-tetradecyltetrahydrothiophen and 2-tridecyltetrahydrothiopyran

Preparation of 1-mesyloxyoctadec-4-ene and its reaction with sodium hydrogen sulphide

Methyl octadec-4-enoate[122] (372 mg, 1.26 mmole) in dry ether (5 ml) was reduced with lithium aluminium hydride (100 mg, 2.63 mmole) in dry ether (10 ml) to give octadec-4-enol (360 mg) which showed a single spot on analytical TLC (PE25) and was used for the next step of reaction without any further purification.

Octadec-4-enol (165 mg) was converted to 1-mesyloxyoctadec-4-ene (177 mg) in the usual way. Its NMR spectrum (CCl_4, 100 MHz) contained the following signals: 4.66 (m, 2H, -CH=CH-), 5.87 (t, 2H, $-CH_2(OSO_2CH_3)$), 7.12 (s, 3H, $-CH_2(OSO_2CH_3)$), 7.95 (m, 4H, $-CH_2CH=CHCH_2-$), 8.23 (m, 2H, $-CH_2CH=CHCH_2CH_2CH_2(OSO_2CH_3)$), 8.74 (br.s, 22H, $-(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-). Its infrared spectrum (liquid film) showed characteristic absorption bands at 1340 and 1170 cm^{-1} (associated with S=O group). There was a complete absence of absorption band at 3500 cm^{-1} (OH).

1-Mesyloxyoctadec-4-ene (168 mg, 0.5 mmole), left at room temperature with a solution of sodium hydrogen sulphide (100 mg, 1.8 mmole) in dimethylformamide (5 ml), afforded a product (148 mg) which showed three bands (A, B, and C) on TLC (PE10).

Band A (43%) with no peak on GLC was considered to be the dimer of 1-mercapto-octadec-4-ene.

After reduction with lithium aluminium hydride this material (26 mg) gave a product (21 mg) which was divided into two portions by prep TLC (PE10). The larger portion (13 mg, ECL 17.8) is probably

1-mercapto-octadec-4-ene since it reacted with acetyl chloride to give a product (ECL 21.2) with a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) in its IR spectrum. The smaller portion (4 mg, ECL almost entirely 18.6) had the same ECL as an authentic sample of 2-tetradecyltetrahydrothiophen (18.6) and was recovered unchanged after treatment with acetyl chloride.

Band B [13%, ECL 17.8 (60%) and 18.6 (40%)*] was considered to be mainly 1-mercapto-octadec-4-ene on the basis of its NMR spectrum (CCl_4, 100 MHz) which contained the following signals: 4.70 (m, 2H, -CH=CH-), 7.30-7.68 (m, 2H, $-CH_2SH$), 7.94 (m, 4H, $-CH_2CH=CHCH_2-$), 8.30 (m, 2H, $-CH_2CH=CHCH_2CH_2CH_2SH$), 8.72 (br.s, ca 23H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-). After acetylation band B had an ECL of 21.2. The infrared spectrum (liquid film) of this acetylated product showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) contained signals at 4.70 (m, 2H, -CH=CH-), 7.20 (t, 2H, $-CH_2(SCOCH_3)-$), 7.74 (s, 3H, $-CH_2(SCOCH_3)-$), 7.96 (m, 4H, $-CH_2CH=CHCH_2-$), 8.41 (m, 2H, $-CH_2CH=CHCH_2CH_2CH_2(SCOCH_3)-$), 8.74 (br.s, 22H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

Band C [30% ECL 18.6] was considered to be 2-tetradecyltetrahydrothiophene. The NMR spectrum (CCl_4, 100 MHz) showed the following signals: 6.66-6.82 (m, 1H, $-CH_2CH(S)CH_2CH_2CH_2$), 7.28 (t, 2H, $-CH_2CH(S)CH_2CH_2CH_2$), 8.04 (m, 4H, $-CH_2CH(S)CH_2CH_2CH_2$), 8.74 (br.s, 26H, $-(CH_2)_n-$) and 9.12τ (t, 3H, CH_3CH_2-).

The mass spectrum showed a molecular ion peak at m/e 289 along with a characteristic fragment of m/e 87 believed to be  . Other fragments have already been reported fully in the Discussion.

*This peak may be due to 2-tetradecyltetrahydrothiophen formed during GLC. Band B showed almost entirely one peak after acetylation (ECL 21.2).

Preparation of 1-mesyloxyoctadec-5-ene and its reaction with sodium hydrogen sulphide

1-Mesyloxyoctadec-5-ene, prepared from octadec-5-enol, in the similar manner showed the following signals in its NMR spectrum (CCl_4, 100 MHz): 4.70 (m, 2H, -CH=CH-), 5.87 (t, 2H, $-CH_2(OSO_2CH_3)$), 7.12 (s, 3H, $-CH_2(OSO_2CH_3)$), 7.97 (m, 4H, $-CH_2CH=CH-CH_2-$), 8.20-8.58 (m, 4H, $-CH_2CH=CHCH_2CH_2CH_2CH_2(OSO_2CH_3)-$), 8.74 (br.s, 20H, $-(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-). Its infrared spectrum (liquid film) showed characteristic absorption bands at 1340 and 1170 cm^{-1} (associated with SO_2 group). There was a complete absence of absorption band at 3500 cm^{-1} (OH).

1-Mesyloxyoctadec-5-ene (153 mg, 0.45 mmole), left at room temperature with a solution of sodium hydrogen sulphide in dimethylformamide (5 ml), gave a product (138 mg) which separated into three bands by prep TLC (PE10).

Band A (60%) showed no peak on GLC and was considered to be the dimer of 1-mercapto-octadec-5-ene.

After reduction with lithium aluminium hydride this material (32 mg) gave a product (27 mg, ECL 17.8) which showed a single spot on TLC. The acetylated product showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) in its infrared spectrum and its NMR spectrum (CCl_4, 100 MHz) contained signals for olefinic protons at 4.70, and a three-proton singlet at 7.76τ along with other usual signals.

Band B [30%, ECL 17.8 (90%) and *18.1 (10%)] was mainly 1-mercapto-octadec-5-ene.

The NMR spectrum (CCl_4, 100 MHz) of band B contained signals at

* The formation of this peak was possibly during GLC, because band B after acetylation showed only one peak (ECL 21.2).

4.70 (m, 2H, -CH=CH-), 7.28-7.68 (m, 2H, $-CH_2SH$), 8.00 (m, 4H, $-CH_2CH=CHCH_2-$), 8.40-8.52 (m, 4H, $-CH_2CH=CHCH_2CH_2CH_2CH_2SH$), *8.74 (br.s, ca 21H, $-(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-). After reacting with acetyl chloride the material in band B showed a single peak on GLC (ECL 21.2), its infrared spectrum (liquid film) contained absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) showed a characteristic three-proton signal at 7.76τ ($SCOCH_3$) and other usual signals.

Band C (ca 3%, ECL 18.1) may be 2-tridecyltetrahydrothiopyran but spectroscopic examination was not possible due to lack of material.

Preparation of 1-acetylmercapto-octadec-4-ene and its reaction with methanolic sulphuric acid

1-Mesyloxyoctadec-4-ene (130 mg, 0.4 mmole) and potassium thiolacetate (100 mg, 0.8 mmole) in dimethylformamide (5 ml) were heated at 100°C for 3 hours. The reaction mixture was cooled, diluted with water, acidified with hydrochloric acid (0.1M) and then extracted with ether (2 x 20 ml). Evaporation of the solvent afforded a product (128 mg) which showed a single spot on TLC (PE10). This component (112 mg, ECL 21.2) was considered to be acetylmercapto-octadec-4-ene. The infrared spectrum (liquid film) showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) contained a three-proton singlet at 7.74τ ($SCOCH_3$) along with other usual signals.

1-Acetylmercapto-octadec-4-ene (93 mg, 0.3 mmole) was heated under reflux with a solution of methanolic sulphuric acid (1M, 5 ml) for 3 hr. The reaction mixture was cooled, diluted with water and

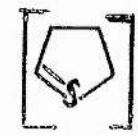
* The single proton -CHSH was hidden by the broad singlet at 8.74τ

then extracted with ether (2 x 10 ml). The product (81 mg) showed two bands A and B on TLC (P100).

Band A [70%, ECL 17.8 (60%), 18.6* (40%)]. After reacting with acetyl chloride, band A showed only one peak on GLC (ECL 21.2). The acetylated product showed an absorption band at 1685 cm^{-1} ($SCOCH_3$) in its infrared spectrum and its NMR spectrum (CCl_4, 100 MHz) contained the characteristic three-proton singlet at 7.74τ ($SCOCH_3$) along with other usual signals.

When a sample of band A (55 mg) was refluxed with a methanolic solution of sulphuric acid (2M, 5 ml) under nitrogen for 6 hours, unreacted material (ca 70%, ECL 17.8) was accompanied by dimer (20%) and by 2-tetradecyltetrahydrothiophen (ca 15%, ECL 18.6).

Band B (25%, ECL 18.6) considered to be 2-tetradecyltetrahydrothiophen . The NMR spectrum (CCl_4, 100 MHz, microcell) contained signals at 6.66-6.82 (m, 1H, $-CH_2CH(S)CH_2CH_2CH_2$), 7.28 (t, 2H, $-CH_2CH(S)CH_2CH_2CH_2$), 8.04 (m, 4H, $-CH_2CH(S)CH_2CH_2CH_2$), 8.74 (br.s, 26H, $-(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-).

The mass spectrum showed a molecular ion peak at m/e 284 along with a characteristic fragment of m/e 87 believed to be  .

Preparation of 1-acetylmercapto-octadec-5-ene and its reaction with methanolic sulphuric acid

1-Mesyloxyoctadec-5-ene (140 mg, 0.45 mmole) and potassium thiolacetate (110 mg, 0.095 mmole) in dimethylformamide (6 ml) were heated at 100°C for 3 hours. The product (128 mg) showed a single spot on TLC (PE10) and this component (115 mg, ECL 21.2) was

* This peak was possibly formed during GLC for the reason already described.

considered to be 1-acetylmercapto-octadec-5-ene. Its infrared spectrum (liquid film) showed a strong absorption band at 1685 cm^{-1} ($SCOCH_3$) and its NMR spectrum (CCl_4, 100 MHz) contained a three-proton singlet at 7.76τ ($SCOCH_3$) along with other usual signals.

1-Acetylmercapto-octadec-5-ene (100 mg, 0.32 mmole, ECL 21.2) was refluxed with methanolic sulphuric acid (1M, 6 ml) for 3 hours. The product [89 mg, ECL 17.8, a single spot on TLC (P)] * was considered to be 1-mercapto-octadec-5-ene. The NMR spectrum (CCl_4, 100 MHz) contained signals at 4.72 (m, 2H, $-CH=CH-$), 7.56 (t, 2H, $-CH_2SH$), 8.00 (m, 4H, $-CH_2CH=CHCH_2-$), 8.24-8.60 (m, 4H, $-CH_2CH=CHCH_2CH_2CH_2CH_2SH$), 8.74 (br.s, ca 21H, $-(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-).

Reaction of 1-mercapto-octadec-5-ene with iodine

1-Mercapto-octadec-5-ene (21 mg) was left at room temperature with a mixture of ethanol and iodine in ethanol (0.05M, 5 ml) for 40 hours. TLC (PE15) of the crude product showed the presence of a non polar spot along with some unchanged starting material and the dimer formed by oxidation with iodine. GLC of the crude product showed peaks of ECL 17.8 (60%) and 18.1 (40%).

The non polar component (5 mg, ECL 18.1) was considered to be 2-tridecyltetrahydrothiopyran . Its NMR spectrum (CCl_4, 100 MHz, microcell) contained signals at 7.38-7.60 (m,1H, $-CH(S)CH_2CH_2CH_2CH_2$), 8.18 (m, 2H, $-CH(S)CH_2CH_2CH_2CH_2$), 8.74 (br.s, 30H, $-(CH_2)_n-$), and 9.12τ (t, 3H, CH_3CH_2-).

The mass spectrum showed a molecular ion peak at m/e 284 and a fragment of m/e 101 believed to be  .

Conversion of 1-mercapto-octadec-4-ene and 5-ene to cyclic sulphur derivatives on standing in air

Samples (10 mg) of 1-mercapto-octadec-4-ene prepared from the mesyloxy alkene by reacting with sodium hydrogen sulphide or via the acetylmercaptoalkene and samples of 1-mercapto-octadec-5-ene also prepared by these two routes were dissolved in ether (5 ml) and kept at room temperature. At daily intervals for seven days aliquots were examined by TLC and GLC. TLC showed the slow formation of dimer and GLC showed the formation of new compounds of ECL 18.6 (from the 4-ene) and 18.1 (from the 5-ene). After one week none of the mercapto alkene remained.

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Part II

The Synthesis of some C_{16} and C_{18} acids of general formula $CH_3(CH_2)_m(CH{=}CH)_nCO_2H$

INTRODUCTION AND DISCUSSION

INTRODUCTION

Commercially available octa-2,4,6-trienoic acid and deca-2,4,6,8-tetraenoic acid have been employed in a study of acyl-CoA synthetases of medium chain length specificity. Their use depends on the change in the UV spectrum of the free acid and its Co-A thiol esters[1]. The position of λ_{max} are given in the table.

	Free acid	CoA thiol ester	Change in λ_{max}
8:3	292 nm	343 nm	51 nm
10:4	323 nm	376 nm	43 nm

These acids suffer from the disadvantage that their activity with long chain acyl Co-A synthetases is low.

It would be advantageous therefore to have C_{16} and C_{18} acids with conjugated triene and tetraene absorption also conjugated with the carbonyl group but such acids are not available from either natural or synthetic sources. The Wittig reaction seemed to promise the greatest chance of success and our synthetic procedures are discussed in the following section. We have further compared the UV spectra of our synthetic acids with those of their thiol esters prepared from dodecyl mercaptan.

DISCUSSION

1. SYNTHETIC PROCEDURES

1.1 Tetradeca-trans-2,trans-4-dienoic acid

Condensation of the Wittig type has been used extensively in the synthesis of compounds related to vitamin A[2] and of other polyunsaturated compounds[3]. The alkene-producing step consists of a condensation between an aldehyde and either a phosphorane or phosphonate. Both the alkyl halide and the aldehyde may be unsaturated and the final product will contain one additional double bond which is usually predominantly trans though, under certain conditions, it may contain increasing proportions of the cis isomer.

Wittig reaction with a phosphorane:

$$RCH_2Br \xrightarrow{Ph_3P} RCH_2\overset{+}{P}Ph_3\overset{-}{Br} \xrightarrow{\text{base}} RCH{=}PPh_3 \longleftrightarrow RC\overset{-}{H}{-}\overset{+}{P}Ph_3 \xrightarrow{R'CHO} RCH{=}CHR'$$

Wittig reaction with a phosphonate:

$$RCH_2Br \xrightarrow{P(OEt)_3} RCH_2P(O)(OEt)_2 \xrightarrow{\text{base}} \overset{-}{R}C\overset{+}{H}P(O)(OEt)_2 \xrightarrow{R'CHO} RCH{=}CHR'$$

The desired C_{14}-dienoate should therefore be available from dodec-2-enal and methyl bromoacetate or from decanal and methyl 4-bromobut-2-enoate. This last compound results from the interaction of methyl crotonate and N-bromosuccinimide. Crombie and Burden[4] recently prepared this dienoate by the phosphonate route. We conducted the experiments described below in an attempt to find methods which could be extended to the C_{16} and C_{18} polyenoates. The sequence of reactions involved is as follows:

Synthesis 1

$$CH_3CH{=}CHCO_2H \xrightarrow{(i)} CH_3CH{=}CHCO_2Me \xrightarrow{(ii)} BrCH_2CH{=}CHCO_2Me$$

$$\xrightarrow{(iii)} (EtO)_2P(O)CH_2CH{=}CHCO_2Me \xrightarrow{(iv)} CH_3(CH_2)_8(CH{=}CH)_2CO_2Me$$

(i) MeOH, H_2SO_4 (ii) N-bromosuccinimide (iii) $(EtO)_3P$

(iv) NaOMe, MeOH or NaH ; $CH_3(CH_2)_8CHO$

When equimolar quantities of methyl 4-diethylphosphonobut-2-enoate and decanal were reacted in dry dimethylformamide in the presence of a slight excess of methanolic sodium methoxide the major product was the aldol resulting from self condensation of decanal. Similar results were obtained when the phosphonate and base were allowed to interact before adding the aldehyde and also in a third experiment conducted in an atmosphere of nitrogen.

A second group of experiments, based on the report of Wadsworth and Emmons[5], was carried out using sodium hydride as the base to convert the phosphonate to its anion using glyme as solvent. At room temperature the desired dienoate (20-25%) was accompanied by a considerable amount of aldol and even less satisfactory results were obtained when the reaction was carried out under reflux. Following Miller and Scrimgeour[6] we also used tetrahydrofuran as solvent at room temperature and obtained the dienoate in 35-40% yield after 3 days and in ~50% yield after extended reaction (2-3 weeks).

Synthesis 2

$$BrCH_2CH{=}CHCO_2Me \xrightarrow{(i)} (C_6H_5)_3\overset{+}{P}CH_2CH{=}CHCO_2MeBr^- \xrightarrow{(ii)}$$

$$(C_6H_5)_3P{=}CHCH{=}CHCO_2Me \xrightarrow{(iii)} CH_3(CH_2)_8(CH{=}CH)_2CO_2Me$$

(i) $(C_6H_5)_3P$ (ii) NaOMe, MeOH (iii) $CH_3(CH_2)_8CHO$

We used the method reported by Bohlmann and Miethe[7] to effect a

conventional Wittig reaction. With a slight excess of methanolic sodium methoxide the major product was again aldol but with half the quantity of base some dienoate (20-25%) resulted. This product contained rather more of the 2t4c isomer (10-15%) than was obtained at the phosphonate reaction but this was reduced to ~5% after iodine isomerisation.

In all these reaction products (1 and 2) there were greater or smaller quantities of aldol (30-40%) along with some unreacted aldehyde (5-10%). The latter was not separated from the dienoate by chromatography but pure dienoic acid was isolated by crystallisation after hydrolysis. By these procedures however the best overall yield was only about 10-15%.

Synthesis 3

$$(C_6H_5)_3\overset{+}{P}CH_2CH{=}CHCO_2Me\,\tilde{B}r \xrightarrow{(i)} (C_6H_5)_3P{=}CHCH{=}CHCO_2Me$$

$$\xrightarrow{(ii)} CH_3(CH_2)_8(CH{=}CH)_2CO_2Me$$

(i) NaOH aqueous (2M) (ii) $CH_3(CH_2)_8CHO$

Gunstone and Barve[8] obtained ethyl octadeca-trans-2-enoate (80%) from palmitaldehyde and carboethoxymethylene triphenyl phosphorane and we have now prepared methyl tetradeca-2,4-dienoate in 80% yield by a similar condensation of decanal and 4-carbomethoxybut-2-enyl triphenyl phosphorane by refluxing in benzene under nitrogen for 12 hours. The recrystallised phosphorane (petrol and benzene mixture), when condensed with aldehyde, gave some product which was not dienoate.

1.2 Hexadeca-trans-2,trans-4-dienoic acid

The Reformatsky reaction is another procedure which has been

extensively used for the synthesis of highly unsaturated compounds such as Vitamin A and related substances. In this reaction an aldehyde or ketone is treated with an α-bromoester and zinc to give, after hydrolysis, a β-hydroxyester, which on subsequent or simultaneous dehydration furnishes an unsaturated ester.

$$BrCH_2CO_2Me + Zn \longrightarrow BrZnCH_2CO_2Me \xrightarrow{RCHO} RCH(OH)CH_2CO_2Me \longrightarrow RCH{=}CHCO_2Me$$

The aldehyde and/or bromoester may be unsaturated and the product, after dehydration, will contain an additional double bond.

Synthesis 4

$$BrCH_2CH{=}CHCO_2Me \xrightarrow{(i)} BrZnCH_2CH{=}CHCO_2Me \xrightarrow{(ii)}$$

$$CH_3(CH_2)_{10}CH(OH)CH_2CH{=}CHCO_2Me \xrightarrow{(iii)} CH_3(CH_2)_{10}CH(OMs)CH_2CH{=}CHCO_2Me$$

$$\xrightarrow{(iv)} CH_3(CH_2)_{10}(CH{=}CH)_2CO_2Me$$

(i) Zn (ii) $CH_3(CH_2)_{10}CHO$ (iii) CH_3SO_2Cl (iv) NaOMe,MeOH; DMSO

Dodecanal (1 mmole) was condensed with methyl 4-bromobut-2-enoate[9] (2 mmoles) in the presence of zinc (2 mmoles) to give methyl 5-hydroxy-hexadec-2-enoate. [The use of a large excess of zinc and of bromoester to improve yields based on the carbonyl compound has been recommended frequently[10,11,12].] Our crude product showed strong OH absorption (3500 cm^{-1}) in its infrared spectrum. TLC showed two spots of low Rf value. Both components were isolated by prep TLC and the more polar one was considered to be the desired hydroxy ester on the basis of its NMR and MS.

Attempts to dehydrate the hydroxy ester with potassium bisulphate, 85% formic acid, anhydrous formic acid, methanolic sulphuric acid (2M, 4M, 10M), iodine in benzene, phosphorus pentoxide, phosphorus

oxychloride, thionyl chloride in pyridine and methyl chlorosulphite all failed. Following Gunstone and Said[13] the hydroxy ester was converted to its mesylate and heated with dry sodium methoxide in dimethyl sulphoxide. The product was acidic and had to be remethylated.

$$CH_3(CH_2)_{10}CH(OH)CH_2CH{=}CHCO_2Me \longrightarrow CH_3(CH_2)_{10}CH(OMs)CH_2CH{=}CHCO_2Me$$

16:2 (2t4t, 2t4c, and 2,5 isomers)

After purification (Prep TLC), GLC showed three major peaks of ECL 19.85 (40%), 19.35 (20%) and 18.7 (30%). After treatment with iodine, peaks were observed at 19.85 (55%) and at 18.7 (25%). It was concluded that the product contained 2,4 and 2,5 dienoates and that this was not a useful procedure for preparing the required conjugated trienoates and tetraenoates.

1.3 Hexadeca and octadeca-trans-2,trans-4,trans-6-trienoic acid

The C_{16} and C_{18} trienoates can be made by Wittig-type reaction by condensing the appropriate aldehyde and bromo ester. In each reaction there is a further choice between the use of Wittig ylide or the phosphonate.

	Aldehyde	
Bromo ester	C_{16}-trienoate	C_{18}-trienoate
$BrCH_2(CH{=}CH)_2CO_2Me$	10:0 (synthesis 5)	12:0 (synthesis 6)
$BrCH_2CH{=}CHCO_2Me$	12:1 (synthesis 7)	[14:1*]
$BrCH_2CO_2Me$	14:2 (synthesis 8)	16:2 (synthesis 9)

* The C_{18}-trienoate was not prepared in this way because later studies showed that it was easier to prepare the dienals for syntheses 8 and 9 than the tetradecenal which would be necessary for this reaction with the phosphorane from methyl 4-bromobut-2-enoate.

Synthesis 5

$$CH_3(CH{=}CH)_2CO_2Me \xrightarrow{(i)} BrCH_2(CH{=}CH)_2CO_2Me \xrightarrow{(ii)}$$

$$(EtO)_2P(O)CH_2(CH{=}CH)_2CO_2Me \xrightarrow{(iii)} (EtO)_2P(O)\bar{C}HCH_2(CH{=}CH)_2CO_2Me$$

$$\xrightarrow{(iv)} CH_3(CH_2)_8(CH{=}CH)_3CO_2Me \xrightarrow{(v)} CH_3(CH_2)_8(CH{=}CH)_3COOH$$

(i) NBS (ii) $(EtO)_3P$ (iii) NaH (iv) $CH_3(CH_2)_8CHO$

(v) KOH,MeOH

Methyl 6-bromohexa-2,4-dienoate was prepared in good yield (60%) by reacting methyl sorbate and NBS without any solvent (when the same reaction was carried out in carbon tetrachloride very little (6%) of the desired bromo ester was obtained). The bromo ester reacted with triethyl phosphite to give the phosphonate and this furnished its anion when treated with sodium hydride. The anion gave the required triene ester by reaction with decanal. After isolation by column chromatography the trienoate (31%) still contained some unreacted aldehyde and gave two major peaks on GLC. These are probably the 2t, 4t, 6t (85%) and 2t,4t,6c (10%) trienoates. Treatment with iodine converts these to a 2t,4t,6t (90%) and 2t,4t,6c (6%) mixture. It is not possible to say whether the product still contains these two isomers or whether it is modified under the condition of the GLC to a mixture of this composition.

Synthesis 6

$$(EtO)_2P(O)CH_2(CH{=}CH)_2CO_2Me \xrightarrow{(i)} (EtO)_2P(O)\bar{C}H(CH{=}CH)_2CO_2Me$$

$$\xrightarrow{(ii)} CH_3(CH_2)_{10}(CH{=}CH)_3CO_2Me \xrightarrow{(iii)} CH_3(CH_2)_{10}(CH{=}CH)_3COOH$$

(i) NaH (ii) $CH_3(CH_2)_{10}CHO$ (iii) KOH,MeOH

Dodecanal was condensed with methyl 6-diethylphosphono-hexa-2,4-dienoate under the same conditions as in Synthesis 5. Isomerisation and purification by column chromatography afforded the trienoate in 30% yield. It gave two peaks on GLC and these are considered to be the 2t,4t,6t (90%) and 2t,4t,6c (7%) isomers.

The C_{16} and C_{18} triene esters were only obtained successfully in this way with due care to two points of experimental detail. (1) The conversion of the phosphonate to its anion is an exothermic process and should be conducted below 20° in an inert atmosphere otherwise the red-brown solution of anion deposits a black solid and there is a poor yield of trienoate. (2) The addition of aldehyde to phosphonate anion should be conducted in dilute solution because of the heavy gummy precipitate which is formed.

Synthesis 7

$$CH_3(CH_2)_8CH{=}CHCHO \xrightarrow{(i)} CH_3(CH_2)_8(CH{=}CH)_3CO_2Me \xrightarrow{(ii)}$$

$$CH_3(CH_2)_8(CH{=}CH)_3CO_2H$$

(i) $Ph_3P{=}CHCH{=}CHCO_2Me$ (ii) KOH,MeOH

Dodec-2-enal (see section 1.5) was condensed with 4-carbomethoxybut-2-enyl triphenyl phosphorane to give the triene ester. Isomerisation and purification by column chromatography furnished the trienoate in 52% yield. GLC showed two major peaks which are probably the 2t,4t,6t (80%) and 2t,4c,6t (3%) isomers.

Previous to this experiment we failed several times to react dodec-2-enal with both the phosphorane and the phosphonate from methyl 4-bromobut-2-enoate, in the presence of either sodium hydride or sodium methoxide. Other workers have reported low yields in Wittig reaction with $\alpha\beta$-unsaturated aldehydes and these have been attributed

to the ease with which such aldehydes undergo aldol condensation and subsequent polymerisation[14,15]. Trippett and Walker[16] successfully condensed 2-enals with phosphoranes and we too found this method to be satisfactory. Dodec-2-enal was converted to the C_{16}-trienoate in 52% yield.

Synthesis 8

$CH_3(CH_2)_8(CH{=}CH)_2CHO \xrightarrow{(i)} CH_3(CH_2)_8(CH{=}CH)_3CO_2Me \xrightarrow{(ii)}$

$CH_3(CH_2)_8(CH{=}CH)_3CO_2H$

(i) $Ph_3P{=}CHCO_2Me$ (ii) KOH,MeOH

Tetradeca-2,4-dienal (see section 1.6) was reacted with carbomethoxymethylene triphenyl phosphorane to give the C_{16}-trienoate. Isomerisation and purification by column chromatography gave methyl hexadeca-2,4,6-trienoate in 76% yield. GLC showed two peaks which are probably the 2t,4t,6t (90%) and 2c,4t,6t (5%) esters.

Synthesis 9

$CH_3(CH_2)_{10}(CH{=}CH)_2CHO \xrightarrow{(i)} CH_3(CH_2)_{10}(CH{=}CH)_3CO_2Me$

$\xrightarrow{(ii)} CH_3(CH_2)_{10}(CH{=}CH)_3COOH$

(i) $Ph_3P{=}CHCO_2Me$ (ii) KOH,MeOH

Hexadeca-2,4-dienal (see section 1.6) was condensed with carbomethoxymethylene triphenyl phosphorane to give the C_{18}-trienoate. Isomerisation and purification by column chromatography gave the trienoic ester in 80% yield. GLC showed two peaks which are probably the 2t,4t,6t (90%) and 2c,4t,6t (4%) isomers.

1.4 Hexadeca and octadeca-trans-2,trans-4,trans-6,trans-8-tetraenoic acid

Following Bergelson et al[17] we have prepared these tetraenoic acids by Wittig condensation between methyl 4-bromobut-2-enoate and an appropriate 2,4-dienal.

Synthesis 10

$CH_3(CH_2)_6(CH{=}CH)_2CHO \xrightarrow{(i)} CH_3(CH_2)_6(CH{=}CH)_4CO_2Me \xrightarrow{(ii)}$

$CH_3(CH_2)_6(CH{=}CH)_4CO_2H$

(i) $Ph_3P{=}CHCH{=}CHCO_2Me$ (ii) KOH,MeOH

Dodeca-2,4-dienal (see section 1.6) was reacted with 4-carbomethoxybut-2-enyl triphenyl phosphorane. Isomerisation and purification gave the required tetraenoate in 46% yield. GLC showed several minor peaks along with the two peaks corresponding to the 2t,4t,6t,8t (80%) and 2t,4c,6t,8t (7%) esters. The tetra-enoate was hydrolysed to acid and re-esterified after purification but GLC showed the same behaviour as before. It is possible that some isomerisation occurs during chromatography[18].

Synthesis 11

$CH_3(CH_2)_8(CH{=}CH)_2CHO \xrightarrow{(i)} CH_3(CH_2)_8(CH{=}CH)_4CO_2Me \xrightarrow{(ii)}$

$CH_3(CH_2)_8(CH{=}CH)_4CO_2H$

(i) $Ph_3P{=}CHCH{=}CHCO_2Me$ (ii) KOH,MeOH

Tetradeca-2,4-dienal, reacted with 4-carbomethoxybut-2-enyl triphenyl phosphorane and subjected to isomerisation and purification, gave tetraenoic ester in 40% yield. GLC showed several minor peaks along with two peaks corresponding to the 2t4t,6t,8t (80%) and

2t,4c,6t,8t (8%) isomers. These esters were hydrolysed, purified and re-esterified but GLC showed the same behaviour. Again we consider that this might be a change during chromatography.

1.5 Dodec-2-enal

The αβ-unsaturated aldehyde required for one of our synthetic procedures was obtained by application of Bedoukian's method[19].

$$CH_3(CH_2)_8CH_2CH_2CHO \xrightarrow{(i)} CH_3(CH_2)_8CH_2CH{=}CHOCOCH_3 \xrightarrow{(ii)}$$

$$CH_3(CH_2)_8CH_2CHBrCHBrOCOCH_3 \xrightarrow{(iii)} CH_3(CH_2)_8CH_2CHBrCH(OMe)_2$$

$$\xrightarrow{(iv)} CH_3(CH_2)_8CH{=}CHCH(OMe)_2 \xrightarrow{(v)} CH_3(CH_2)_8CH{=}CHCHO$$

(i) Ac_2O,KOAc (ii) Br_2,CCl_4 (iii) MeOH (iv) KOH,BuOH
(v) $(HO_2CCH_2)_2C(OH)CO_2H$ (citric acid)

These reactions proceeded smoothly but the αβ-unsaturated aldehydes were accompanied by a second product which Bedoukian considered to be ketene acetal. Purification was effected by column chromatography and dodec-2-enal obtained in an overall yield of 10-15%. The additional component, after purification, was shown to be methyl dodecanoate by IR, NMR and MS. The formation of such a product can be explained by the fact that ketene acetals readily form addition compounds in the presence of water and alcohol[20] under acidic conditions.

1.6 Dodeca-, tetradeca-, and hexadeca-2,4-dienals

We prepared these 2,4-dienals by the procedure reported by Pippen and Nonaka[21] for the C_6-C_{12} members.

$$CH_3(CH_2)_nCHO \xrightarrow{(i)} CH_3(CH_2)_nCH(OH)C{\equiv}CCH{=}CHOCH_3 \xrightarrow{(ii)}$$

$$CH_3(CH_2)_nCH(OH)CH{=}CHCH{=}CHOCH_3 \xrightarrow{(iii)} CH_3(CH_2)_nCH{=}CHCH{=}CHCHO$$

[n = 8, 10 or 12]

(i) $BrMgC{\equiv}CCH{=}CHOCH_3$ (ii) $LiAlH_4$ (iii) H_2SO_4 acid (2M)

1-Methoxybut-1-en-3-yne was condensed with the appropriate saturated aldehyde and the product, treated first with lithium aluminium hydride[22] and then with acid, gave the dienal in ~60-70% yield. The C_{12} and C_{14} compounds were greenish-yellow oils, the C_{16} dienal was a pale yellow low-melting solid.

This procedure had the following advantages over other methods of preparing 2,4-dienals[23,24,25]; the starting materials are readily available; all steps may be carried out in one flask, without isolation of intermediates; the product is easy to purify; and yields are good.

2. PROPERTIES OF THE UNSATURATED ALDEHYDES, ACIDS, AND ESTERS

2.1 ECL of unsaturated aldehydes and esters

Our observed ECL are collected in Table 1. There is some chromatographic separation of the various cis trans isomers and the all-trans forms appear to have the highest ECL. The detailed structure of the partial cis compounds is not fully known in all cases.

Table 1 ECL of unsaturated aldehydes and esters

Chain length	No. of double bonds			
	1 (2t)	2 (2t4t)		
aldehydes				
C_{12}	13.50	16.00		
C_{14}	-	17.90		
C_{16}	-	19.85		
	2 (2t4t)	3 (2t4t6t)	4 (2t4t6t8t)	
esters				
C_{14}	17.80	-	-	
C_{16}	19.90	22.30	24.40	
C_{18}	-	24.20	26.30	
	2t4c	2t4t6c	2c4t6t	2t4c6t8t
C_{14}	16.80	-	-	-
C_{16}	19.40	21.90	21.80	24.00
C_{18}	-	23.70	23.70	25.90

2.2 Nuclear magnetic resonance spectroscopy

The several unsaturated acids prepared in this project along with some unsaturated aldehydes required as intermediates have been examined

by NMR. With the assistance of some publications[3,4,26-36] dealing with the NMR spectra of related compounds it has been possible to provide a reasonable correlation of our own results.

$CH_3(CH_2)_nCH_2(CH{=}CH)_mCH{=}CHX$ (X = $COOCH_3$ or CHO)

Signals for $C\underline{H}_3$, $(C\underline{H}_2)_n$, $C\underline{H}_2(CH{=}CH)_m$, $COOC\underline{H}_3$ and $C\underline{H}O$ are present with the expected chemical shifts and the only signals of interest are those resulting from the olefinic protons. These fall into three groups: the proton attached to C(2), the proton attached to C(3) and the remaining protons whose signals could not be distinguished from each other.

Of these the C(3) proton has the lowest τ value and appears as a double doublet with J values of 10 Hz and 15 Hz and the C(2) proton has the highest τ value and appears as a doublet (J = 15 Hz) for the ester and as a double doublet (J = 16 Hz and 9 Hz) for the aldehyde. The remaining olefinic protons give signals close to those of the C(2) protons and appear as a multiplet which increases in complexity with the number of protons. These conclusions have been confirmed in the case of methyl tetradeca-trans-2,trans-4-dienoate by decoupling experiments carried out in the presence of $Eu(fod)_3$ as a chemical shift reagent. In the presence of this shift reagent, signals were observed as in Table 2.

Table 2 NMR signals (τ) of 14:2 in the presence and absence of $Eu(fod)_3$

$Eu(fod)_3$	C(2)	C(3)	C(4)	C(5)	C(6)
absent	4.32	2.86	3.88 to 3.96		7.78
present	0.88	-0.60	3.13	3.90	7.81

When irradiated at 7.8τ the signal for the C(5) proton appeared as a doublet ($J_{4,5}$ = 15 Hz) and that for the C(4) proton as a double doublet ($J_{4,5}$ = 15 Hz, $J_{3,4}$ = 10 Hz). Irradiation at -0.6τ converted the signal for the C(2) proton into a singlet and C(4) proton appeared as a double doublet ($J_{4,5}$ = 15 Hz, $J_{2,4}$ = 10 Hz) through coupling with C(5) and C(2) protons.

These coupling constants confirm that both double bonds are trans.

2.3 Mass spectra

(a) Methyl esters The mass spectra of 14:2, 16:3, 18:3, 16:4 and 18:4 esters (see section 11) provide some interesting correlations which assist in the interpretation of the results. All spectra show the following peaks:

(i) Peaks for M, M-31 ($-OCH_3$), and M-59 ($-COOCH_3$).

(ii) A series of peaks for fragments of the type $-(CH_2)_n(CH{=}CH)_mCO_2CH_3$ along with further fragmentation resulting from loss of 32 mass units as set out in the following table.

Esters

	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}
14:2	111*	125*	139*	153*			
16:3	111*		137*	151*	165*	179	
18:3	111		137	151	165*	179	193
16:4	111†		137†		163*	177*	
18:4	111†		137†		163*	177*	

* Peaks at 32 mass units less are also present

† Only peaks at 32 mass units less are present

(iii) Unidentified peaks include one at m/e 91 in all spectra (possibly the tropylium ion, $C_7H_7^+$), a peak at m/e 113 in the diene ester and peaks at m/e 149 and 117 in the tetraenes only.

(iv) The peaks at m/e 138 in the two trienes is possibly $[H(CH{=}CH)_3CO_2CH_3]^+$ which has been reported previously in the mass spectra of the non-conjugated 6,9,12 and 9,12,15-octadecatrienoates. This fragment may lose a further 32 mass units to provide a peak at m/e 107.

(v) The base peak is at m/e 91 for two tetraenes and at 79 for the diene and both triene esters.

These spectra provide further confirmation that in all spectra the unsaturated system is completely conjugated and starts at C(2).

(b) Thiol esters. These spectra are characterised by the following peaks:

(i) In all four esters the base peak is due to the cleavage of between the carbonyl group and the sulphur atom.

(ii) There are major peaks at m/e 147 and 133 in the tetraene esters and at 107, 105 and 91 in all esters. These may be due

to the tropylium ion (91) and to other fragments based on this unit viz. 91 + 14 (CH_2), 91 + 16 (O), 91 + 26 (-CH=CH-) and 91 + 26 + 14, the last two being confined to the tetraene esters.

2.4 Ultraviolet spectroscopy

We report the ultraviolet spectroscopic characteristics of our conjugated aldehydes, acids, esters and thiol esters which are collected in table 3. The results show that λ_{max} of the C_{16} and C_{18} trienoic acid and tetraenoic acids are increased by 32 nm and 28 nm respectively when their thiol esters are formed. It is expected therefore that they should be useful for the study of acyl-CoA synthetases of long chain length specificity.

Table 3 Ultraviolet absorption of unsaturated aldehydes, acids, esters and thiol esters

$CH_3(CH_2)_n(CH{=}CH)_mX$, X = -CHO, $-CO_2H$, $-COS(CH_2)_{11}CH_3$

Chain length	acids	methyl esters	thiol esters
14:2	-	263 (33.40)	-
16:2	-	263 (-)**	-
16:3	298 (40.20)	303 (40.30)	330 (24.13)
18:3	298 (40.32)	302 (40.52)	330 (23.90)
16:4	333 (40.23)	325	360 (26.95)
18:4	331 (40.54)	333	360 (37.89)

also the following aldehydes: 12:1 [223 nm (16.60)]; 14:2 [274 nm (33.00)]; and 16:2 [275 nm (34.54)].

* Values quoted are λ_{max} nm (EtOH solution) and ϵ_{max} (x 10^{-3})

** The conjugated dienoate was not isolated in a pure state.

3. Conclusion

Our objective has been the preparation of the C_{16} and C_{18} triene (Δ2, 4, 6) and tetraene (Δ2, 4, 6, 8) acids. After examination of several routes we found Wittig condensation between a dienal (RCH=CHCH=CHCHO) and an isolated phosphorane (from $BrCH_2COOCH_3$ and $BrCH_2CH=CHCOOCH_3$) to be the most satisfactory (75-80%).

These acids were converted into the thiol esters of dodecyl mercaptan and the UV spectra of the acids and thiol esters compared. Esterification resulted in a shift of λ_{max} by 32 nm for the trienes and 28 nm for the tetraenes. This is an encouraging result and the value of these unsaturated acids for the study of long-chain acyl Co-A synthetase is being undertaken elsewhere.

EXPERIMENTAL

General Chemical Procedures*

Isomerisation

The isolated ester containing mainly all-trans isomer along with some cis compound(s) was added to a solution of iodine in carbon tetrachloride (0.05M) and left in the daylight for 1-3 days. The excess of iodine was removed with aqueous dilute thiosulphate solution (2M) and the organic product recovered.

1. Methyl tetradeca-trans-2,trans-4-dienoate

Synthesis 1

$$CH_3CH{=}CHCO_2H \xrightarrow{(i)} CH_3CH{=}CHCO_2Me \xrightarrow{(ii)} BrCH_2CH{=}CHCO_2Me$$

$$\xrightarrow{(iii)} (EtO)_2P(O)CH_2CH{=}CHCO_2Me \xrightarrow{(iv)} CH_3(CH_2)_8(CH{=}CH)_2CO_2Me$$

(i) $MeOH,H_2SO_4$ (ii) NBS (iii) $(EtO)_3P$ (iv) $NaH;CH_3(CH_2)_8CHO$

Methyl crotonate

Crotonic acid (150 g) was refluxed with methanolic sulphuric acid (0.45M, 250 ml) for 1 hour. The mixture was poured into water (400 ml) saturated with sodium chloride and extracted with ether (2 x 200 ml) to yield methyl crotonate (143 g, b.p. 30-32°/30 mm, lit.[37] 42-44°/36 mm).

Methyl γ-bromocrotonate[38,39,40]

Methyl crotonate (46.6 g, 0.46 mmole), N-bromosuccinimide (57.0 g, 0.32 mmole) and benzoyl peroxide (0.2 g) were placed in a three-necked round-bottomed flask (500 ml) fitted with a stirrer, nitrogen inlet tube and reflux condenser. The reaction mixture was stirred and refluxed in a nitrogen atmosphere for 2 hours. The precipitated succinimide was removed by suction filtration,

* More general chemical procedures are reported in the experimental section of Part 1 of this thesis.

washed (2 x 30 ml) with carbon tetrachloride and the carbon tetrachloride washings combined with the filtrate. After removal of solvent the residue was fractionally distilled *in vacuo* through a column (30 cm long, 10 mm outside diameter) packed with glass helices. Nitrogen was led into the capillary and after a small forerun (2-3 g), methyl γ-bromocrotonate (32.3 g, 58%, b.p. 38-42°/0.25 mm; lit.[41] 78-81°/8 mm) was collected.

Methyl γ-diethylphosphonocrotonate[4,42]

Triethyl phosphite (5 g, 0.03 mmole) was maintained at 110° (oil bath) and methyl γ-bromocrotonate (5.4 g, 0.03 mmole) was added dropwise to cause gentle reflux. When the addition was complete, the mixture was heated to 150-160°C for 30 minutes. Distillation gave methyl γ-diethylphosphonocrotonate (5.6 g, 75%, b.p. 118-134°/0.4 mm; lit.[4] 115-130°/0.3 mm).

Methyl tetradeca-trans-2,trans-4-dienoate[5,43]

A slurry of sodium hydride (50%, 96 mg, 2 mmole) in dry 1,2-dimethoxyethane (10 ml) was cooled to 20° and stirred during dropwise addition of methyl γ-diethylphosphonocrotonate (472 mg, 2 mmole). After the addition, the solution was stirred at room temperature until the gas evolution had ceased (1 hour). The reddish-brown solution was maintained below 20-25° during dropwise addition of decanal (312 mg, 2 mmole) and then stirred at room temperature for 1 hour and refluxed for 30 minutes. After cooling, a large excess of water was added and the product (513 mg) was extracted with ether and purified by prep TLC (PE10). The methyl tetradeca-*trans*-2,*trans*-4-dienoate (132 mg, 31%) was shown to be relatively pure by GLC [ECL 16.8 (~6%, 2t4c) and 17.8 (~90%, 2t4t); after hydrogenation the GLC showed only a single peak for methyl myristate (ECL 14.0)]. The

ultraviolet spectrum showed an absorption maximum at 263 nm (33,400) and the infrared spectrum contained absorption bands at 1720 (ester), 1620 (C=C) and 1000 cm^{-1} (CH=CH). The NMR spectrum showed signals at 2.86 (dd, 1H, $-\underline{CH}=CHCOOCH_3$), 3.88-3.96 (m, 2H, $-\underline{CH}=\underline{CH}CH=CHCOOCH_3$), 4.32 (d, 1H, $-CH=\underline{CH}COOCH_3$), 6.36 (S, 3H, $-COO\underline{CH}_3$), 7.78 (dt, 2H, $-\underline{CH}_2(CH=CH)_2$), 8.74 (br.s, 14H, $-(\underline{CH}_2)_7-$) and 9.12τ (t, 3H, $\underline{CH}_3(CH_2)_8-$).

Synthesis 2

$$BrCH_2CH=CHCO_2Me \xrightarrow{(i)} (C_6H_5)_3\overset{+}{P}CH_2CH=CHCO_2Me\,\overset{-}{Br} \xrightarrow{(ii)}$$

$$(C_6H_5)_3\overset{+}{P}\text{-}\overset{-}{C}HCH=CHCO_2Me \xrightarrow{(iii)} CH_3(CH_2)_8(CH=CH)_2CO_2Me$$

(i) $(C_6H_5)_3P$ (ii) NaOMe,MeOH (iii) $CH_3(CH_2)_8CHO$

4-Carbomethoxybut-2-enyl triphenyl phosphonium bromide[44]

Triphenyl phosphine (44.5 g, 0.17 mmole) dissolved in dry benzene (200 ml) was stirred at room temperature during the addition (20 min) of methyl γ-bromocrotonate (25 g, 0.14 mmole). The resulting suspension was stirred overnight at ambient temperature and filtered through a sintered funnel. The colourless crystals were washed with benzene and petrol ether and dried over P_2O_5 under vacuum (60 g, 85%, m.p. 154-155°).

Methyl tetradeca-trans-2,trans-4-dienoate[9,45]

4-Carbomethoxybut-2-enyl triphenyl phosphonium bromide (3.25 g, 7.5 mmole) was dissolved in dry methanol (15 ml) and methanolic sodium methoxide (6 mmole in 2ml methanol) was added dropwise during an hour with vigorous stirring. The orange solution was stirred at room temperature for another hour under nitrogen. After the dropwise addition (30 min) of decanal (1.1 g, 7.00 mmole), the reaction mixture was refluxed for 3-4 hours under nitrogen. The solvent was

evaporated under vacuum and ether was added to extract the dienoate. Insoluble triphenyl phosphine oxide was filtered off. After the removal of ether the residue was again treated with petrol ether and some further triphenyl phosphine oxide was filtered off. After isomerisation (iodine in carbon tetrachloride, 0.05M, 30 ml), the crude product was purified by prep TLC (PE10). Methyl tetradeca-trans-2,trans-4-dienoate (295 mg, 21%) was shown to be pure by GLC [ECL 16.80 (7%, 2t4c) and 17.80 (85%, 2t4t), hydrogenation gave methyl myristate (ECL 14.0) as the only product]. λ_{max} 263 nm (33,200). The IR spectrum contained absorption bands at 1720 (ester), 1620 (C=C) and 1000 cm^{-1} ($CH^{t}=CH$). The NMR spectrum showed the following signals: 2.53 (dd, 1H, $-\underline{CH}=CHCOOCH_3$), 3.88-4.04 (m, 2H, $-\underline{CH}=\underline{CH}CH=CHCOOCH_3$), 4.34 (d, 1H, $-CH=\underline{CH}COOCH_3$), 6.36 (s, 3H, $-COOC\underline{H}_3$), 7.78 (dt, 2H, $-C\underline{H}_2(CH=CH)_2$), 8.74 (br.s, 14H, $-(C\underline{H}_2)_7-$) and 9.12τ (t, 3H, $C\underline{H}_3(CH_2)_8-$).

Synthesis 3

$$(C_6H_5)_3\overset{+}{P}CH_2CH=CHCO_2Me\,\overset{-}{Br} \xrightarrow{(i)} (C_6H_5)_3P=CHCH=CHCO_2Me$$

$$\xrightarrow{(ii)} CH_3(CH_2)_8(CH=CH)_2CO_2Me$$

(i) NaOH aqueous (2M) (ii) $CH_3(CH_2)_8CHO$

4-Carbomethoxybut-2-enyl triphenyl phosphorane*

4-Carbomethoxybut-2-enyl triphenyl phosphonium bromide (8.82 g, 0.02 mmole) was dissolved in water and dilute aqueous sodium hydroxide (2M) was added dropwise, while stirring, until the supernatant water showed a pink colour with phenolphthalein. The precipitated salt (6.85 g, 93%, m.p. 142-143°) was filtered through a sintered funnel, washed with water and dried over P_2O_5 under vacuum.

* Attempts to prepare 6-carbomethoxyhexa-2,4-dienyl phosphorane by a similar procedure were unsuccessful. The heavy orange-coloured precipitate changed to a black powder on drying.

Methyl tetradeca-trans-2,trans-4-dienoate

Decanal (700 mg, 4.5 mmole) and 4-carbomethoxybut-2-enyl triphenyl phosphorane (2.5 g, 5mmole excess) were refluxed in dry benzene (30 ml) under nitrogen for 12 hours. The solvent was evaporated under vacuum and ether was added to dissolve the dienoate. The suspension was filtered to remove insoluble triphenyl phosphine oxide and the ether was evaporated. The residue was again dissolved in petrol ether and the small amount of triphenyl phosphine oxide which separated was filtered off. The crude product (1.3 g) after isomerisation (iodine in carbon tetrachloride, 0.05M, 50 ml) was purified by column chromatography. The methyl tetradeca-trans-2, trans-4-dienoate which was eluted mainly by PE10 (0.8 g, 80%) was contaminated with a small amount of unreacted aldehyde. This was removed by converting the ester into acid and crystallising it from petrol ether. The re-esterified material was shown to be pure by GLC [ECL 16.8 (6%, 2t4c) and 17.82 (80%, 2t4t), hydrogenation gave methyl myristate (ECL 14.0) as the only product]. It showed ultraviolet absorption at λ_{max} 263 nm (33,100). The IR spectrum contained absorption bands at 1720 (ester), 1620 (C=C) and 1000 cm^{-1} ($CH=^{t}CH$). The NMR signals were at 2.86 (dd, 1H, $-\underline{CH}=CHCOOCH_3$), 3.88-3.96 (m, 2H, $-\underline{CH}=\underline{CH}CH=CHCOOCH_3$), 4.32 (d, 1H, $-CH=\underline{CH}COOCH_3$), 6.36 (s, 3H, $-COOC\underline{H}_3$), 7.78 (dt, 2H, $-\underline{CH}_2(CH=CH)_2$), 8.47 (br.2, 14H, $-(\underline{CH}_2)_7-$), and 9.12τ (t, 3H, $C\underline{H}_3(CH_2)_8-$).

2. Methyl hexadeca-trans-2,trans-4-dienoate.

Synthesis 4

$BrCH_2CH=CHCO_2Me \xrightarrow{(i)} BrZnCH_2CH=CHCO_2Me \xrightarrow{(ii)}$

$CH_3(CH_2)_{10}CH(OH)CH_2CH=CHCO_2Me \xrightarrow{(iii)} CH_3(CH_2)_{10}CH(OSO_2Me)CH_2CH=CHCO_2Me$

$\xrightarrow{(iv)} CH_3(CH_2)_{10}CH=CHCH=CHCO_2Me$

(i) Zn (ii) $CH_3(CH_2)_{10}CHO$ (iii) CH_3SO_2Cl (iv) NaOMe,MeOH;DMSO

Methyl 5-hydroxyhexadec-2-enoate[46,47] *

A mixture of methyl bromocrotonate (1 g, 0.056 mmole) and decanal (1 g, 0.054 mmole), dissolved in dry benzene (5 ml) and dry ether (1 ml), was placed in a dropping funnel. Granulated zinc (0.36 g, 0.056 mmole, freshly cleaned with hydrochloric acid and dried with absolute ethanol and sodium dry ether) was placed in the reaction flask.

Approximately one-tenth of the mixture was run into the flask and a few crystals of iodine were added. The reaction began when the flask was warmed and stirring was continued during the slow addition of the remainder of the reactants over 25 minutes. The mixture was then maintained at reflux temperature for an additional 3 hours. After cooling, the stirred mixture was acidified with sulphuric acid (2M, 10 ml) with vigorous stirring. Twenty minutes later, the organic layer was extracted with ether, washed with aqueous sulphuric acid (1M), aqueous sodium carbonate (2M) and twice with water. Evaporation of the solvent afforded a yellow oily liquid (2.2 g) with strong infrared absorption at 3500 cm^{-1} (OH). TLC showed two major spots at low Rf, one of which was methyl 5-hydroxyhexadec-2-enoate [ECL 25.9, TMS derivative 18.4]. Hydrogenation of this fraction gave a mixture of methyl palmitate (ECL 16.0), methyl 5-oxopalmitate (ECL 22.4) and methyl 5-hydroxypalmitate (ECL 25.0).

Attempts to prepare methyl hexadeca-trans-2,trans-4-dienoate from the product of the Reformatsky reaction.

(i) Attempts were made to dehydrate the crude hydroxy ester by heating the hydroxy ester with fused potassium bisulphate[48],

* Attempts to condense dodec-2-enal and methyl 6-bromohexa-2,4-dienoate by the Reformatsky reaction procedure were unsuccessful.

85% formic acid[48], anhydrous formic acid[50,51,52], methanolic sulphuric acid (2M, 5M, and 10M)[53], or by refluxing a benzene solution of hydroxy ester with iodine[54], phosphorous pentoxide[55], phosphorous oxychloride[55,56] or thionyl chloride and pyridine[57]. The product, examined by TLC and UV, showed no evidence of dehydration.

(ii) Freshly prepared methyl chlorosulphite[58] (5 ml) was added dropwise to a solution of crude hydroxy ester in pyridine (1 ml) and tetrahydrofuran (10 ml) maintained at -10° to -5°C. The reaction mixture was allowed to warm up to room temperature during 4 hours. Water (10 ml) and hydrochloric acid (2M, 5 ml) were then added and the product extracted with ether. The crude product was examined by TLC (PE10) and UV, but there was no evidence of any conjugated diene ester.

(iii) TLC pure methyl 5-hydroxyhexadeca-trans-2-enoate was converted to its mesyloxy ester by the usual method (see Part I) and this (60 mg, 0.16 mmole) was heated at 100°C with dry sodium methoxide in dry dimethylsulphoxide (3 ml) for 4 hours[13]. After the addition of water, followed by acidification with hydrochloric acid (2M), the aqueous layer was extracted with ether. The product which showed acidic behaviour on a TLC plate was re-esterified [dry methanol (2 ml), 14% BF_3,MeOH (0.5 ml)] and the major components were isolated by prep TLC (PE10, 37 mg). After treatment with a solution of iodine in carbon tetrachloride (0.05M, 5 ml, in daylight for 2 days) the product showed major peaks at 19.4 (20%, 2t4c), 19.9 (40%, 2t4t) and 18.7 (30%, this may be non-conjugated diene) by GLC. The isomerised product showed the expected UV absorption at 263 nm.

3. Hexadeca-trans-2,trans-4,trans-6-trienoic acid

Synthesis 5

$$CH_3(CH{=}CH)_2CO_2Me \xrightarrow{(i)} BrCH_2(CH{=}CH)_2CO_2Me \xrightarrow{(ii)}$$
$$(EtO)_2P(O)CH_2(CH{=}CH)_2CO_2Me \xrightarrow{(iii)} (EtO)_2\overset{+}{P}(O)\overset{-}{C}H(CH{=}CH)_2CO_2Me$$
$$\xrightarrow{(iv)} CH_3(CH_2)_8(CH{=}CH)_3CO_2Me \xrightarrow{(v)} CH_3(CH_2)_8(CH{=}CH)_3CO_2H$$

(i) NBS (ii) $(EtO)_3P$ (iii) NaH (iv) $CH_3(CH_2)_8CHO$
(v) KOH,MeOH

Methyl 6-bromohexa-2,4-dienoate[59,60] *

A mixture of powdered N-bromosuccinimide (54 g, 0.3 mmole), methyl sorbate (152 g, 1.2 mmole, b.p. 36-38°/0.4 mm) and benzoyl peroxide (0.5 g) was stirred at 120° (oil bath) under nitrogen until the bulk of the bromoimide had dissolved. After a further 15 minutes at this temperature, the mixture was cooled and the separated succinimide was filtered off and washed with carbon tetrachloride. The filtrate, washed once with sodium carbonate (2M) and twice with water, gave methyl 6-bromohexa-2,4-dienoate (42 g, 60%, b.p. 76-84°/0.5 mm; lit.[59] 75°/1 mm).

[When carbon tetrachloride was used as solvent, the yield was as low as 6%[61].]

Methyl 6-diethylphosphonhexa-2,4-dienoate

Triethyl phosphite (23.5 g, 0.17 mole) was maintained at

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* Attempt to prepare methyl 8-bromoocta-2,4,6-trienoate

A solution of methyl octa-2,4,6-trienoate (5 g, 0.033 mmole) and N-bromosuccinimide (1.5 g, 0.008 mmole) in carbon tetrachloride (150 ml) was refluxed under nitrogen for 3 hours. The product (5.5 g) was mainly unreacted trienoate accompanied by some non-polar material which was not methyl 8-bromoocta-2,4,6-trienoate on the basis of its NMR.

110° (oil bath) and methyl 6-bromohexa-2,4-dienoate (35 g, 0.17 mmole) was added dropwise to cause gentle reflux. When the addition was complete, the mixture was heated at 150-160°C for 30 minutes. Distillation under nitrogen gave methyl 6-diethylphophon-hexa-2,4-dienoate (25 g, 60%, b.p. 126-142°/0.5 mm).

Methyl hexadeca-trans-2,trans-4,trans-6-trienoate[6]

To a slurry of sodium hydride (50%, 1.54 g, 0.032 mmole) in dry tetrahydrofuran (100 ml) at 20°C, methyl 6-diethylphosphonohexa-2,4-dienoate (7.7 g, 0.032 mmole), was added with stirring during 30 minutes. Stirring was continued at room temperature for a further hour until the gas evolution had ceased. To the reddish-brown solution, decanal (5 g, 0.032, in 10 ml dry tetrahydrofuran) was added during 1 hour. During the addition, a gummy precipitate appeared. The solution was stirred at room temperature for 3-4 days. Thereafter, a large excess of water was added and the product was extracted with ether and dried.

The crude product (7.1 g) was treated with iodine (iodine in carbon tetrachloride, 0.05M, 50 ml, in daylight for 2-3 days). The solvent was evaporated and the excess of iodine was removed by washing with aqueous sodium thiosulphate. The ether extracted product (7 g) was purified by column chromatography (M-60 sorbsil). The trienoate (2.6 g, 31%)*, which was eluted mainly by PE7.5 and PE10, contained ~6% of the 2t4t6c isomer (ECL 21.8), 85% of the 2t4t6t isomer (ECL 22.3), and a trace of decanal. After crystallising from petrol the triene ester showed UV absorption at 303 nm (40,300) and its infrared spectrum contained absorption bands at 1720 (ester), 1648 and 1620 (C=C) and 1000 cm^{-1} ($CH^{t}=CH$). The NMR spectrum

* Another experiment under nitrogen using glyme as solvent gave methyl hexadeca-trans-2,trans-4,trans-6-trienoate in 20-25% yield.

showed signals at 2.83 (dd, 1H, -CH=CHCOOCH$_3$), 3.42-4.12 (m, 4H, -(CH=CH)$_2$CH=CHCOOCH$_3$), 4.28 (d, 1H, CH=CHCOOCH$_3$), 6.38 (s, 3H, COOCH$_3$), 7.78 (dt, 2H, CH$_2$(CH=CH)$_3$), 8.74 (br.s, 14H, -(CH$_2$)$_7$-), and 9.12τ (t, 3H, CH$_3$(CH$_2$)$_8$-).

Hexadeca-trans-2,trans-4,trans-6-trienoic acid

Methyl hexadeca-trans-2,trans-4,trans-6-trienoate (2.5 g) was refluxed with potassium hydroxide (1.0 g) in methanol (10 ml) for 1 hour. After acidification (1M HCl) and cooling the acid (2.2 g) was isolated as waxy crystals. Crystallisation from petrol ether at low temperature (-5 to -10°C) gave hexadeca-trans-2,trans-4, trans-6-trienoic acid [m.p. 85-86°C, λ_{max} 298 nm (40,200)].

4. Octadeca-trans-2,trans-4,trans-6-trienoic acid

Synthesis 6

$$(EtO)_2P(O)CH_2(CH=CH)_2CO_2Me \xrightarrow{(i)} (EtO)_2\overset{+}{P}(O)\ddot{C}H(CH=CH)_2CO_2Me$$

$$\xrightarrow{(ii)} CH_3(CH_2)_{10}(CH=CH)_3CO_2Me \xrightarrow{(iii)} CH_3(CH_2)_{10}(CH=CH)_3CO_2H$$

(i) NaH (ii) $CH_3(CH_2)_{10}CHO$ (iii) KOH,MeOH

Methyl octadeca-trans-2,trans-4,trans-6-trienoate

Methyl 6-diethylphosphonohexa-2,4-dienoate (7.08 g, 0.03 mmole) and dodecanal (5.52 g, 0.03 mmole) were condensed under the same conditions as described in synthesis 5. The product (7.5 g), after isomerisation, was purified by column chromatography and the triene ester, together with a little unreacted aldehyde, was eluted mainly by PE7.5 and PE10. The methyl octadeca-trans-2,trans-4,trans-6-trienoate (3.6 g, 30%) contained 7% of the 2t4t6c isomer (ECL 23.7)

and 90% of the 2t4t6t isomer (ECL 24.2). [After hydrogenation, GLC showed a single peak of methyl stearate (ECL 18.0).] The ultraviolet spectrum showed a peak at λ_{max} 302 nm (40,520). The IR spectrum (nujol mull) contained bands at 1720 (ester), 1645 and 1620 (C=C) and 1005 cm^{-1} (CH=CH). The NMR spectrum (CCl_4, 100 MHz) showed the same signals as the C_{16}-trienoate except that it had 18 protons at 8.74τ.

Octadeca-trans-2,-trans-4,trans-6-trienoic acid

Methyl octadeca-trans-2,trans-4,trans-6-trienoate (3. g) was refluxed with potassium hydroxide (1.5 g) in methanol for 1 hour. The crude acid, recovered as pale yellow waxy crystals, was crystallised from petrol ether to give pure octadeca-trans-2,trans-4, trans-6-trienoic acid [m.p. 81-82°, λ_{max} 298 nm (40,230)].

5. Hexadeca-trans-2,trans-4,trans-6-trienoic acid

Synthesis 7

$$CH_3(CH_2)_8CH{=}CHCHO \xrightarrow{(i)} CH_3(CH_2)_8(CH{=}CH)_3CO_2Me \xrightarrow{(ii)}$$

$$CH_3(CH_2)_8(CH{=}CH)_3CO_2H$$

(i) $Ph_3P{=}CHCH{=}CHCO_2Me$ (ii) KOH,MeOH

Preparation of enolacetate[19]

Dodecanal (97 g, 0.53 mmole), acetic anhydride (134.15 g, 1,3 mmole, freshly distilled) and potassium acetate (10 g) were refluxed for one hour. After cooling, the reaction mixture was washed with water and with sodium carbonate solution (0.5M). The resultant oil was distilled and the enolacetate obtained in relatively pure form. (44 g, 37.5%, b.p. 116-118/1.5 mm; lit.[19] 111-113/3mm). NMR (CCl_4, 100 MHz) resonances were present at 2.96-3.08 (dd, 1H, -CH=CHOCOCH$_3$),

4.60-4.86 (m, 1H, $-CH_2-\underline{CH}=CHOCOCH_3$), 7.94-8.02 (dt, 2H, $-\underline{CH_2}CH=CHOCOCH_3$), 7.98 (s, 3H, $-CH=CHOCOC\underline{H_3}$), 8.74 (br.s, 16H, $-(\underline{CH_2})_8-$) and 9.12τ (t, 3H, $\underline{CH_3}(CH_2)_9-$).

Bromination of enolacetate

Bromine (10 ml, 28 g) in an equal volume of carbon tetrachloride was added slowly to a stirred solution of the enolacetate (43 g, 0.18 mmole) in carbon tetrachloride (50 ml) over 20-30 minutes. The temperature was not allowed to rise above 10°C.

Formation of bromoacetal

The brominated reaction mixture was added to dry methanol (125 ml) with stirring and cooling and allowed to stand for two days with occasional shaking. After addition of water (1 l.) the separated oil was washed with sodium carbonate solution (0.5M) until free of acid and then fractionally distilled in the presence of a small amount of sodium carbonate (68 g, 87.5%, b.p. 140-146°/1.5 mm; lit.[19] 151-153°/4 mm). The NMR (CCl_4, 100 MHz) resonances were observed at 5.70 [d, 1H, $-CHBrC\underline{H}(OMe)_2$], 6.18 [m, 1H, $-\underline{CH}BrCH(OMe)_2$], 6.66 and 6.68 [S, 6H, $-CH(OC\underline{H_3})_2$], 8.74 [br.s, 16H, $-(\underline{CH_2})_8-$] and 9.12τ [t, 3H, $\underline{CH_3}(CH_2)_9-$].

Preparation of the acetal of dodecenal

The bromoacetal (42 g, 0.13 mmole) was added to a solution of potassium hydroxide (14.5 g, 0.26 mmole) in methanol (70 ml) and butanol (70 ml). The mixture (which formed two layers) was gently heated and the methanol allowed to distil off. When the butanol began to distil off, the mixture was refluxed for 1 hour during which time potassium bromide precipitated. At the end of 1 hour the mixture was washed with water and the oil fractionally distilled in the presence of several pellets of potassium hydroxide to give the acetal (18 g,

b.p. 116-135°/1.5 mm; lit.[19] 95-97°/2 mm). TLC showed only one spot and GLC showed one major peak (ECL 12.0). The NMR (CCl_4, 100 MHz) resonances were present at 4.20-4.42 [m, 2H, $-CH_2\underline{C}H=CHCH(OCH_3)_2$], 4.62-4.72 [dd, 1H, $-CH_2\cdot CH=\underline{C}HCH(OCH_3)_2$], 5.38 [d, 1H, $-CH_2CH=CH\underline{C}H(OCH_3)_2$], 6.84 [s, 6H, $-CH(OC\underline{H}_3)_2$], 7.74-8.10 [dt, 2H, $-C\underline{H}_2CH=CH-$], 8.74 [br.s, 14H, $-(C\underline{H}_2)_7-$] and 9.12τ [t, 3H, $C\underline{H}_3(CH_2)_8-$]. The IR (liquid film) contained absorption bands at 1640 (-C=C) and 970 cm^{-1} ($CH=\overset{t}{C}H$).

Preparation of dodec-2-enal

The acetal (8.5 g, 0.037 mmole) was boiled with an equimolar amount of citric acid (7.10 g in aqueous methanol (50%) in an open vessel until the escaping vapour reached a temperature of 95°C. The product, after washing with sodium carbonate solution (0.5M), was distilled to give dodec-2-enal (6.2 g, 62%, b.p. 107-112°/1.5 mm; lit.[19] b.p. 125-126°/10 mm).

Purification was effected in a column of silica (M-60, sorbsil) the aldehyde being eluted with PE8 [almost entirely a single peak on GLC (ECL 13.5)]. It showed IR absorption bands at 2720 (CHO), 1680 (C=O), 1630 (C=C) and 965 cm^{-1} ($CH=\overset{t}{C}H$) and UV absorption at 223 nm (16,000). The NMR spectrum (CCl_4, 100 MHz) showed the following signals: 0.60 (d, 1H, $=CHC\underline{H}O$), 3.30 (dt, 1H, $-C\underline{H}=CHCHO$), 4.00 (dd, 1H, $CH=C\underline{H}CHO$), 7.60-7.80 (dt, 2H, $-C\underline{H}_2CH=CH-$), 8.72 (br.s, 14H, $-(C\underline{H}_2)_7-$) and 9.12τ (t, 3H, $C\underline{H}_3(CH_2)_8-$).

Methyl hexadeca-trans-2,trans-4,trans-6-trienoate

Dodec-2-enal (1 g, 5.5 mmole) was condensed with 4-carbomethoxy-but-2-enyl triphenyl phosphorane (2.16 g, 6 mmole) in refluxing benzene under nitrogen by the procedure described in synthesis 3. Crude trienoate (1.7 g) was purified by column chromatography followed by crystallisation. The pure hexadeca-trans-2,trans-4,trans-6-trienoate

[(750 mg, 62%, ECL 21.9 (2t4c6t, 8%) and 22.3 (2t4t6t, 80%), after hydrogenation, the GLC showed only a single peak for methyl palmitate (ECL 16.0)]. λ_{max} of the trienoate was at 303 nm (40,270). The IR (nujol mull) absorption bands were at 1720 (ester), 1640 (C=C) and 1005 cm^{-1} ($CH^{t}=CH$). The NMR (CCl_4, 100 MHz) spectrum contained the following signals: 2.83 (dd, 1H, $-C\underline{H}=CHCOOCH_3$), 3.42-4.12 (m, 4H, $-(C\underline{H}=C\underline{H})_2CH=CHCOOCH_3$), 4.28 (d, 1H, $-CH=C\underline{H}COOCH_3$), 6.36 (s, 3H, $COOC\underline{H}_3$), 7.78 (dt, 2H, $C\underline{H}_2CH=CH-$), 8.74 (br.s, 14H, $-(C\underline{H}_2)_7-$) and 9.12τ (t, 3H, $C\underline{H}_3(CH_2)_8-$).

Hexadeca-trans-2,trans-4,trans-6-trienoic acid

The triene ester (500 mg) was refluxed with potassium hydroxide (250 mg) in methanol (5 ml). After adding water and hydrochloric acid (1M) the trienoic acid was extracted with ether and crystallised from petrol (m.p. 84-85° , λ_{max} 298 nm (40,230)).

6. Hexadeca-trans-2,trans-4,trans-6-trienoic acid

Synthesis 8

$$CH_3(CH_2)_8(CH=CH)_2CHO \xrightarrow{(i)} CH_3(CH_2)_8(CH=CH)_3CO_2Me \xrightarrow{(ii)} CH_3(CH_2)_8(CH=CH)_3CO_2H$$

(i) $Ph_3P=CHCO_2Me$ (ii) KOH,MeOH

Starting materials

Commercially available 1-methoxybut-1-en-3-yne was obtained as a 50% solution in aqueous methanol. To recover the enyne, ether was added and the whole mixture was thoroughly washed with water several times, dried and then distilled under reduced pressure (b.p. 28°/12 mm; lit.[62] b.p. 42°/15 mm; lit.[63] b.p. 29-33°/16 mm).

Ethyl bromide was dried over calcium chloride and redistilled.

Decanal and dodecanal, purchased from BDH Chemical Co Ltd, Poole, Dorset, were washed with saturated sodium carbonate solution and dried.

Commercially available tetrahydrofuran was kept over $LiAlH_4$ and distilled from fresh hydride immediately before use.

Tetradeca-2,4-dienal[21,64,65]

A solution of 1-methoxybut-1-en-3-yne (10.66 g, 0.13 mmole) in tetrahydrofuran (70 ml) was added with stirring to a Grignard reagent[66] [from magnesium (2.92 g, 0.12 mmole) and ethyl bromide (3.08 g, 0.12 mmole) in tetrahydrofuran (80 ml)] maintained at about 40°C. After an additional hour of stirring at room temperature, the reaction flask was cooled and a solution of dodecanal (18.72 g, 0.12 mmole) in tetrahydrofuran (30 ml) was added over 30 minutes. After two hours of stirring at room temperature, the mixture was cooled and absolute ethanol (5.52 g, 0.12 mmole) was added. Thirty minutes later, lithium aluminium hydride (4.56 g, 0.12 mmole) was added in small proportions at intervals of 20-30 minutes. The mixture was stirred for 2 hours at room temperature and left overnight. It was then treated successively with ethyl acetate (6 ml), water (30 ml), and aqueous sulphuric acid (2M, 150 ml). The organic layer was separated and the aqueous layer was re-extracted with more ether. After washing with sodium carbonate solution (1M) and three times with water the combined ether solutions were dried over anhydrous sodium sulphate. After evaporation of the solvent the residue was fractionally distilled in vacuo to get pure tetradeca-trans-2,trans-4, dienal [14.8 g, 68%, b.p. 140-142°/1.5 mm]. The aldehyde was a greenish yellow oil [GLC showed a single peak of ECL 17.9, λ_{max} 274 nm (33,100). The infrared absorption contained bands at

2720 (CHO), 1680 (C=O), 1620 (-C=C-) and 990 cm^{-1} ($CH^{t}=CH$).] The NMR (CCl_4, 100 MHz) resonances were at 0.60 (d, 1H, C$\underline{H}$O), 3.05 (dd, 1H, -C$\underline{H}$=CHCHO), 3.74-3.88 (m, 2H, -C$\underline{H}$=C$\underline{H}$CH=CHCHO), 4.06 (dd, 1H, =C$\underline{H}$CHO), 7.78 (dt, 2H, -C$\underline{H}_2$(CH=CH)$_2$), 8.65 (br.s, 14H, -(C$\underline{H}_2$)$_7$-) and 9.12 τ (t, 3H, C$\underline{H}_3$(CH$_2$)$_8$-).

Carbomethoxymethyl triphenyl phosphonium bromide

Triphenyl phosphine (40 g, 0.25 mmole) and methyl bromoacetate (31.5 g, 0.22 mmole) were treated by the method already described. The phosphonium bromide (74 g, 80%) was obtained on colourless waxy crystals.

Carbomethoxymethylene triphenyl phosphorane

Carbomethoxymethyl triphenyl phosphonium bromide (39.5 g, 0.1 mmole) was dissolved in water and dilute aqueous sodium hydroxide was added dropwise with stirring, until the supernatant water showed a pink colour with phenolphthalein. The precipitate was filtered through a sintered funnel, washed with water and dried over P_2O_5 under vacuum [30 g, 95%, m.p. 164-165°C, lit.[44] 162-163°C).

Methyl hexadeca-trans-2,trans-4,trans-6-trienoate

Tetradeca-trans-2,trans-4-dienal(4.57 g, 0.022 mmole) and carbomethoxymethylene triphenyl phosphorane (7.87 g, 0.025 mmole) were refluxed in benzene solution under nitrogen for 12 hours. The crude trienoate was recovered by the usual method, isomerised (iodine in carbon tetrachloride 0.05M, 30 ml) and purified by column chromatography using petrol, and diethyl ether as the eluting solvent. The trienoate (4.42 g, 76%), eluted mainly by PE10, was substantially pure [ECL 21.8 (5%, 2c4t6t) and 22.3 (90%, 2t4t6t)]. Hydrogenation gave methyl palmitate (ECL 16.0) as the only product. λ_{max} 302 nm (40,480). The NMR resonances were at 2.83 (dd, 1H, -C$\underline{H}$=CHCOOCH$_3$),

3.42-4.12 (m, 4H, $-(CH=CH)_2CH=CHCOOCH_3$), 4.28 (d, 1H, $-CH=CHCOOCH_3$), 6.36 (s, 3H, $-COOCH_3$), 7.76 (dt, 2H, $-CH_2(CH=CH)_2^-$), 8.76 (br.s, 14H, $-(CH_2)_7-$), and 9.12τ (t, 3H, $CH_3(CH_2)_8-$).

Hexadeca-trans-2,trans-4,trans-6-trienoic acid

The ester (1 g) was refluxed with potassium hydroxide (0.5 g) in methanol (10 ml) for 1 hour and the acid isolated as waxy crystals (850 mg). Crystallisation from petrol at low temperature (-5° to $-10^{\circ}C$) gave hexadeca-trans-2,trans-4,trans-6-trienoic acid [m.p. $84-85^{\circ}C$, λ_{max} 298 (40,200)].

7. Octadeca-trans-2,trans-4-trans-6-trienoic acid

Synthesis 9

$$CH_3(CH_2)_{10}(CH=CH)_2CHO \xrightarrow{(i)} CH_3(CH_2)_{10}(CH=CH)_3CO_2Me \xrightarrow{(ii)}$$

$$CH_3(CH_2)_{10}(CH=CH)_3CO_2H$$

(i) $Ph_3P=CHCO_2Me$ (ii) KOH,MeOH

Hexadeca-2,4-dienal

This dienal was prepared in 80% yield by the procedure described for the C_{14}-dienal. It was a reddish yellow solid and was purified by column chromatography, being eluted mainly by PE7.5 and PE10. [GLC showed a single peak of ECL 19.85; λ_{max} 274.5 nm (34,540)]. The IR spectrum contained bands at 2725 (CHO), 1685 (HCO), 1625 (-C=C-) and 1000 cm^{-1} $(CH^{t}=CH)_2$. The NMR (CCl_4, 100 MHz) resonances were at 0.60 (d, 1H, $-CHO$), 3.03 (dd, 1H, $-CH=CHCHO$), 3.76-3.86 (m, 2H, $-CH=CHCH=CHCHO$), 4.02 (dd, 1H, $-CH=CHCHO$), 7.78 (dt, 2H, $-CH_2(CH=CH)_2^-$), 8.65 (br.s, 18H, $-(CH_2)_9-$) and 9.12τ (t, 3H, $CH_3(CH_2)_{10}-$).

Methyl octadeca-trans-2,trans-4,trans-6-trienoate

Hexadeca-2,4-dienal (7.08 g, 0.030 mmole) was treated with carbomethoxymethylene triphenyl phosphorane (10.62 g, 0.032 mmole) by the same procedure as before. The crude product (8.82 g), after isomerisation (iodine in carbon tetrachloride, 0.05M, 50 ml), was purified by column chromatography (7.7 g, 80%). The trienoate was shown to be pure by GLC [ECL 23.7 (4%, 2c4t6t) and 24.2 (90%, 2t4t6t); hydrogenation gave only methyl stearate (ECL 18.0)]. λ_{max} 302 nm (40,520). The NMR (CCl_4, 100 MHz) spectrum contained signals at 2.83 (dd, 1H, $-\underline{CH}=CHCOOCH_3$), 3.42-4.14 (m, 4H, $-(\underline{CH}=\underline{CH})_2CH=CHCOOCH_3$), 4.28 (d, 1H, $-CH=\underline{CH}COOCH_3$), 6.36 (s, 3H, $-COOC\underline{H}_3$), 7.78 (dt, 2H, $-\underline{CH}_2(CH=CH)-_2$), 8.74 (br.s, 18H, $-(\underline{CH}_2)_9-$), and 9.12τ (t, 3H, $\underline{CH}_3(CH_2)_{10}-$).

Octadeca-trans-2,trans-4,trans-6-trienoic acid

Methyl octadeca-trans-2,trans-4,trans-6-trienoate (3 g) was refluxed with potassium hydroxide (1.5 g) in methanol (15 ml) for 1 hour, and the trienoic acid isolated as pale yellow waxy crystals (2.5 g). Crystallisation from light petrol at low temperature (-5 to -10°C) gave octadeca-trans-2,trans-4,trans-6-trienoic acid [m.p. 81-82°C, λ_{max} 298 (40,320)].

8. Hexadeca-trans-2,trans-4,trans-6,trans-8-tetraenoic acid

Synthesis 10

$$CH_3(CH_2)_6(CH=CH)_2CHO \xrightarrow{(i)} CH_3(CH_2)_6(CH=CH)_4CO_2Me \xrightarrow{(ii)} CH_3(CH_2)_6(CH=CH)_4CO_2H$$

(i) $Ph_3P=CHCH=CHCO_2Me$ (ii) KOH, MeOH

Dodeca-trans-2,trans-4-dienal

This aldehyde was prepared in 55% yield (b.p. 98-100°/0.5 mm; lit.[21] b.p. 134-136°/8 mm) by the same procedure as before.

Its ultraviolet spectrum showed λ_{max} at 275 nm (33,000) and its IR (liquid film) spectrum contained bands at 2720 (CHO), 1685 (HCO), 1620 (-C=C-) and 1000 cm^{-1} $(CH{=}^{t}CH)_2$. GLC showed a single peak of ECL 16.0. The NMR spectrum showed the following signals: 0.60 (d, 1H, -CHO), 3.07 (dd, 1H, -CH=CHCHO), 3.76-3.84 (m, 2H, -CH=CHCH=CHCHO), 4.04 (dd, 1H, -CH=CHCHO), 7.78 (dt, 2H, $-CH_2(CH{=}CH)_2^-$), 8.65 (br.s, 10H, $(CH_2)_5$-), and 9.12τ (t, 3H, $CH_3(CH_2)_6$-).

Methyl hexadeca-trans-2,trans-4,trans-6,trans-8-tetraenoic acid

Dodeca-trans-2,trans-4-dienal (5 g; 0.028 mmole) was condensed with 4-carbomethoxybut-2-enyl triphenyl phosphorane (10 g, 0.028 mmole) in refluxing benzene (100 ml) under nitrogen for 12 hours. The product after isomerisation with iodine (iodine in carbon tetrachloride, 50 ml) was purified by column chromatography (M-60, sorbsil). The tetraenoate, eluted mainly by PE10, showed a single spot on TLC (PE10). [3.29 , 46%, λ_{max} 335 nm (40,320)]. The ester was purified by crystallisation from petrol but GLC of the crystallised product showed several peaks [ECL 18.45, 18.95, 20.00, 23.67, 24.0 (7%), and 24.4 (80%); hydrogenation of the product gave only methyl palmitate] The NMR (CCl_4, 100 MHz) resonances were at 2.82 (dd, $-CH{=}CHCOOCH_3$), 3.16-4.18 (m, 7H, $-(CH{=}CH)_3CH{=}CHCOOCH_3$), 6.36 (s, 3H, $-COOCH_3$), 7.78 (dt, 2H, $-CH_2(CH{=}CH)_4^-$), 8.74 (br.s, 10H, $(CH_2)_5$-) and 9.12τ (t, 3H, $CH_3(CH_2)_6$-).

Hexadeca-trans-2,trans-4,trans-6,trans-8-tetraenoic acid

The tetraenoate (1.0 g) was refluxed with potassium hydroxide (0.4 g) in methanol (10 ml). The mixture was cooled at ice-bath,

diluted with water, acidified with hydrochloric acid (1M, 5-10 ml) and a brownish yellow solid was extracted with ether (2 x 20 ml). Crystallisation from petrol at low temperature (-5 to -10°C) gave pure acid [pale brown crystals, m.p. 132-134°, λ_{max} 333 nm (40,230)].

9. Octadeca-trans-2,trans-4,trans-6,trans-8-tetraenoic acid

Synthesis 11

$CH_3(CH_2)_8(CH{=}CH)_2CHO \xrightarrow{(i)} CH_3(CH_2)_8(CH{=}CH)_4CO_2Me \xrightarrow{(ii)}$

$CH_3(CH_2)_8(CH{=}CH)_4CO_2H$

(i) $Ph_3P{=}CHCH{=}CHCO_2Me$ (ii) KOH,MeOH

Methyl octadeca-trans-2,trans-4,trans-6,trans-8-tetraenoate

Tetradeca-2,4-dienal (5 g, 0.024 mmole) and 4-carbomethoxybut-2-enyl triphenyl phosphorane (8.68 g, 0.024 mmole) were refluxed in dry benzene under nitrogen for 12 hours. The resultant tetraenoate (6.8 g) was isomerised with iodine (iodine in carbon tetrachloride, 0.05 , 50 ml) and purification was effected by column chromatography (M-60, sorbsil). The tetraenoate was eluted mainly by PE10 (2.82 g, 40%). TLC (PE10) showed a single spot but there were several peaks on GLC [ECL 19.5, 20.8, 21.71, 25.45, 25.9 (8%) and 26.3 (80%); hydrogenation gave only methyl stearate (ECL 18.0)]. λ_{max} 333 nm (40,270). The NMR (CCl_4, 100 MHz) resonances were at 2.83 (dd, 1H, $-\underline{CH}{=}CHCOOCH_3$), 3.26-4.34 (m, 7H, $-(\underline{CH}{=}\underline{CH})_3CH{=}\underline{CH}COOCH_3$), 6.36 (s, 3H, $-COOC\underline{H}_3$), 7.78 (t, 2H, $-\underline{CH}_2(CH{=}CH)_2$), 8.76 (br.s, 14H, $-(\underline{CH}_2)_7-$), and 9.12τ (t, 3H, $C\underline{H}_3(CH_2)_8-$).

Octadeca-trans-2,trans-4,trans-6,trans-8-tetraenoic acid

Hydrolysis of the ester in the usual way gave the tetraenoic acid [m.p. 111-112°, λ_{max} 331 nm (41,540)].

10. Preparation of thiol esters[67-73]

The acids were slowly mixed with an excess of freshly distilled oxalyl chloride (0.8 mmole) and then refluxed for one hour with exclusion of moisture. The unreacted oxalyl chloride was removed by heating under nitrogen and finally by blowing with nitrogen.

Equimolar amounts of acid chloride and dodecyl mercaptan were heated at 100°C for 1 hour under nitrogen. The thiol ester, isolated in 60-70% yield by prep TLC (PE5), had UV absorption maxima at 380 nm (trienoic thiol esters) and 360 nm (tetraenoic thiol esters). The structure of each thiol ester was confirmed by mass spectra (see section 2).

11. Mass spectra

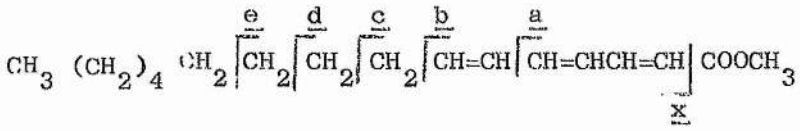
With the help of certain publications[74-92] dealing with the mass spectra of polyunsaturated compounds, we have been able to interpret the mass spectra of our synthetic conjugated methyl di-, tri-, and tetraenoates and some of their thiol esters. The major peaks (m/e) are reported along with peak-intensity relative to the base peak (100). Spectra were recorded at 70 ev.

1. Methyl tetradeca-2,4-dienoate

$$CH_3(CH_2)_4\underset{\underline{e}}{\lceil}CH_2\underset{\underline{d}}{\lceil}CH_2\underset{\underline{c}}{\lceil}CH_2\underset{\underline{b}}{\lceil}CH_2\underset{\underline{a}}{\lceil}CH{=}CHCH{=}CH\underset{\underline{x}}{\rfloor}COOCH_3$$

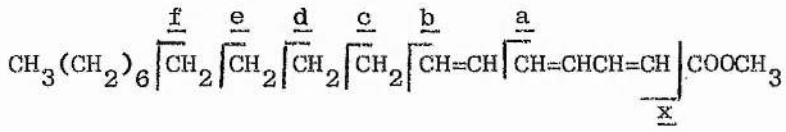
Peaks at: 238 (M, 5), 207 (M-31, 7), 179 (x, 1), 178 (x-1, 2), 177 (x-2, 1), 153 (d, 4), 139 (c, 6), 135 (e-32, 6), 125 (b, 6), 121 (d-32, 9), 113 (?, 41), 111 (a, 90), 107 (c-32, 16), 93 (b-32, 20), 91 (?, 18), 81 (113-32, 72), 79 (a-32, 100) and at 82, 80, 74, 69, 67, 66, 65 and 55 (all > 20).

2. Methyl hexadeca-2,4,6-trienoate



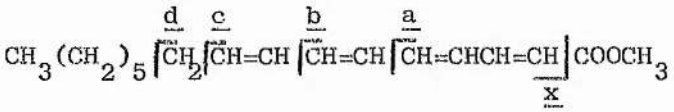
Peaks at: 264 (M, 18), 233 (M-31, 7), 205 (x, 13), 179 (e, 2), 165 (d, 4), 151 (c, 18), 138 (b+1, 49), 137 (b, 18), 133 (d-32, 18), 119 (c-32, 35), 111 (a, 25), 107 (138-31, 38), 105 (b-32, 41), 91 (?, 91), 79 (a-32, 100) and at 99, 82, 81, 78, 77, 71, 67, 59, 57 and 55 (all > 20).

3. Methyl octadeca-2,4,6-trienoate



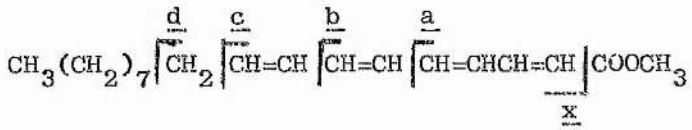
Peaks at: 293 (M, 3), 261 (M-31, 2), 233 (x, 1), 232 (x-1, 1), 193 (f, 1), 179, e, 1), 165 (d, 1), 152 (c+1, 3), 151 (c, 4), 138 (b+1, 24), 137 (b, 8), 133 (d-32, 5), 119 (c-32, 22), 111 (a, 15), 107 (138-31, 22), 105 (b-32, 20), 91 (?, 71), 79 (a-32, 100) and at 82, 81, 78, 77, 71, 67, 59, 57 and 55 (all > 20).

4. Methyl hexadeca,2,4,6,8-tetraenoate



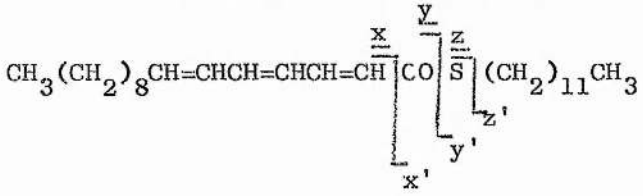
Peaks at: 262 (M, 30), 231 (M-31, 5), 203 (x, 10), 177 (d, 18), 163 (c, 24), 149 (?, 20), 145 (d-32, 20), 131 (c-32, 37), 117 (149-32, 80), 105 (b-32, 75), 91 (?, 100), 79 (a-32, 48) and at 98, 95, 83, 81, 78, 77, 71, 67, 59, 57, and 55 (all > 20).

5. Methyl octadeca-2,4,6,8-tetraenoate



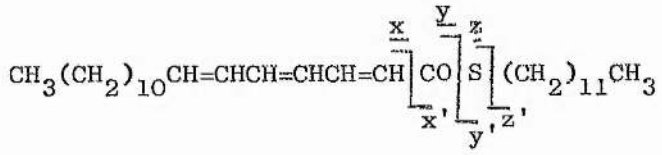
Peaks at: 262 (M, 30), 231 (M-31, 5), 203 (x, 10), 177 (d, 8), 163 (c, 24), 149 (?, 20), 145 (d-32, 16), 131 (c-32, 35), 117 (149-32, 72), 105 (b-32, 95), 91 (?, 100), 79 (a-32, 60) and at 83, 81, 79, 78, 77, 71, 69, 57, and 55 (all > 20).

6. The dodecylmercapto ester of hexadeca-2,4,6-trienoic acid



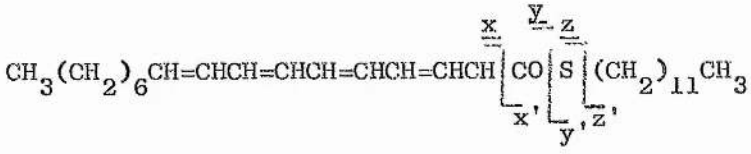
Peaks at: 434 (M, 2), 265 (z, 1), 234 (y+1, 29), 233 (y, 100), 205 (x, 4), 107 (91+16, 95), 91 (C_7H_7, 12) and at 97, 95, 85, 84, 83, 81, 74, 71, 70, 69, 67, 59, 57, 56, and at 55 (all > 20).

7. The dodecylmercaptoester of octadeca-2,4,6-trienoic acid



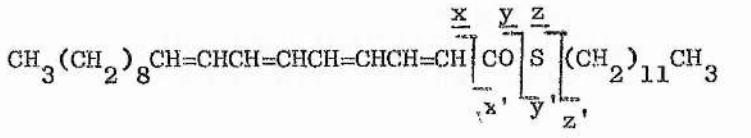
Peaks at: 462 (M, 3), 293 (z, 3), 262 (y+1, 55), 261 (y, 100), 233 (x, 9), 121 (?, 25), 108 (107+1, 33), 107 (91+16, 95), 105 (91+14, 22), 91 (C_7H_7, 56), and at 97, 95, 93, 92, 85, 83, 81, 80, 79, 77, 71, 70, 69, 67, 57, 56, and at 55 (all > 20).

8. The dodecylmercapto ester of hexadeca-2,4,5,8-tetraenoic acid



Peaks at: 432 (M, 17), 263 (z, 3), 232 (y+1, 37), 231 (y, 100), 203 (x, 18), 147 (133+14, 14), 133 (107+26, 86), 117 (?, 24), 107 (91+16, 88), 105 (1+14, 36), 91 (C_7H_7, 56) and at 83, 81, 79, 77, 71, 69, 67, 57, 56, and 55 (all > 20).

9. The dodecylmercapto ester of octadeca-2,4,5,8-tetraenoic acid



Peaks at: 460 (M, 12), 291 (z, 1), 260 (y+1, 38), 259 (y, 100), 231 (x, 23), 147 (133+14, 20), 133 (167+26, 92), 117 (?, 40), 107 (91+16, 97), 105 (91+14, 43), 91 (C_7H_7, 70), and at 83, 81, 79, 69, 67, 57, and 55 (all > 20).

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